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PRINCIPAL INVESTIGATOR: Eva Balint, M.D.

CONTRACTING ORGANIZATION: Stanford University
Stanford, CA 94305

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14. ABSTRACT
This is a multidisciplinary postdoctoral award investigating the role of vitamin D in aromatase inhibitor-induced osteoporosis in breast cancer, supporting studies in basic science, clinical research and epidemiology. During the reporting period, we intensified our efforts to recruit subjects to the clinical trial at Stanford Cancer Center. In addition, we explored collaborations with other academic institutions and local oncology practices to boost enrollment. Unfortunately, the collaborations did not materialize due to financial and time constraints of the award. The trial recently had to be closed due to low recruitment and no analysis can be done on the limited data. A revised statement of work has been submitted and accepted by the DoD in which SEER-Medicare dataset outcomes analysis replaced the basic science component of the award.

15. SUBJECT TERMS
Vitamin D, breast cancer, bone loss, aromatase, inhibitors

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Introduction:

This is a multidisciplinary postdoctoral award investigating the role of vitamin D in preventing aromatase inhibitor-induced osteoporosis in breast cancer. Building on the recipient's past experience in medicine, basic science and bone biology, the award supports the recipient's transition from basic science research towards establishing her as a successful new translational investigator in the breast cancer field. In the current form of the Statement of Work, the award supports outcomes analysis, a prospective clinical trial and education toward a master's degree in the filed of epidemiology. The outcomes analysis component focuses on the SEER-Medicare linked dataset. The clinical research component is a randomized controlled prospective trial of vitamin D in preventing aromatase inhibitor-induced osteoporosis in breast cancer patients. The epidemiology component focuses on the role of vitamin D in breast cancer and prevention of aromatase inhibitor-induced osteoporosis, using methods of epidemiology, and supports the recipient's studies towards obtaining a Master of Science (MS) degree in Clinical Epidemiology.

Body:

Task 1: Obtain vitamin D tablets and verify vitamin D_3 content by tandem mass spectrometry (months 1-2)

This task has been completed. These are the vitamin D study drug capsules administered to study subjects in the randomized controlled clinical trial (Task 5). Vitamin D is considered a dietary supplement and manufacturing is not very tightly regulated. Thus, it is difficult to obtain vitamin D capsules with consistent and reliable active drug content as there is significant variation in vitamin D content of commercially available vitamin D preparations. Vitamin D study drug capsules were obtained from Vital Nutrients (http://www.vitalnutrients.net/), a leader in the dietary supplement manufacturing field. We worked very close with Enrico Liva, RPh, Director of Quality Assurance and his team to achieve the quality and consistency of manufacturing necessary to conform with FDA requirements. Vital Nutrients modified their usual manufacturing practices to generate vitamin D capsules with accurate and consistent vitamin D content necessary to conduct a clinical trial.

Task 2: **Task 8.** Outcomes analysis (months 37-48-)

a. Obtain SEER-Medicare dataset
b. Outcomes analysis

This task has not been completed due to early termination of the award. This is a new task in the Statement of Work approved for the no cost extension period. We started focusing on the outcomes analysis after the new Statement of Work and the No Cost Extension has been approved by the DoD in December 2010. We started searching for a statistician once the funds became available. We initially explored working with statisticians within Stanford University however subsequently we were directed to the

Northern California Cancer Center. The recipient was working closely with Dr. Dee West at the Northern California Cancer Center to locate a statistician with experience with the SEER-Medicare linked dataset and within our budget. Unfortunately, the recipient was not able to contract the statistician due to the early termination of the award. There are no results to report.

Task 3. Final analysis and manuscript preparation of SEER-Medicare data (months 37-48)

This task has not been completed due to early termination of the award. The analysis has not been completed so there are no results to report.

Task 4: Subject enrollment to clinical trial and data collection (months 3-34)

a. Subjects enrolled to clinical trial (50 subjects), initial blood tests and imaging studies (months 3-22)
b. Data collection with bi-monthly visits, blood draws to assess bone turnover parameters and pain assessment, with up to 1-year follow-up (months 3-34)
c. Bi-monthly evaluations of individual sensitivity to vitamin D_3 doses, decisions regarding escalating vitamin D_3 dose on an individual basis
d. Ongoing data entry
e. Interim clinical trial data analysis

This task has been completed. The clinical trial is closed.
This is a clinical trial investigating the effect of higher than currently recommended dose of vitamin D for the prevention of bone loss associated with aromatase inhibitor use for breast cancer in the adjuvant setting. This study is closed for recruitment due to low enrollment. The data collected are not sufficient for analysis.

During the reporting period, we intensified our recruitment efforts at Stanford Cancer Center. We presented the significance of the research and discussed challenges in recruitment at the Breast Cancer Research Group at the Stanford Cancer Center. The oncologists were very committed to the trial however they commented that the trial has been surprisingly difficult to recruit. Please see a list of barriers and factors contributing to low recruitment below.

1. Subject-related factors:

a. Geographical distance/time to travel: Stanford Cancer Center Breast Oncology Clinic serves a large geographic area and many patients travel long distances. Once their primary therapy is finished, patients often receive radiation therapy at their local facility and some return to their local oncologists. This clinical trial recruits at the time adjuvant aromatase inhibitor is started and geography is a limiting factor for many patients as they are not willing to return for their screening and study follow-up visits.

b. Not willing to take either 800 or 2400 IU vitamin D and not willing to be randomized: maybe this has been the most surprising and concerning barrier to recruitment. Due to wide spread media coverage and publicity of the vitamin D controversy

coupled with lack of reliable evidence, patients and physicians take their stand and strongly believe in various vitamin D doses. Most patients we interviewed have been taking vitamin D 400-1000 IU daily (ranging between 0 up to 10,000 IU daily) and some are unwilling to change their regimen. Some patients believe the experimental dose in this clinical trial is either too low or too high, depending where they fall on the spectrum, and they are unwilling to be randomized to either arm. This is most concerning because the subject's strong believes limits the possibility to conduct investigation in this area.

c. Clinical trial fatigue of subjects: This trial recruits subjects at the time when adjuvant therapy is starting. Stanford Cancer Center conducts many clinical trials around diagnosis, various chemotherapy regimens, radiation therapy regimens and supportive and alternative modalities. Patients are approached by clinical trial coordinators multiple times during their treatment at Stanford. Although this trial is not in direct competition with any other trials conducted at the Stanford Cancer Center, it is fairly common for patients to experience trial fatigue and not wanting to participate in a study when nearing the end of their primary treatment.

2. Inclusion/exclusion criteria related factors:

Non-eligible for enrollment. The main reason why potential subjects do not meet eligibility criteria are elevated urine calcium and/or osteoporosis detected by DEXA. About one third of consented participants screen out because of osteoporosis detected on DEXA or elevated urine calcium. Exclusion due to these factors is much higher than previously anticipated. Elevated baseline urinary calcium excretion is likely related to significant bone loss even in subjects who do not meet criteria of osteoporosis on DEXA. In this study urinary calcium is used as an important safety monitoring tool. To date, no study was performed to assess baseline urinary calcium in perimenopausal or postmenopausal women and the effect of 2400 IU vitamin D on urinary calcium excretion has not been studied in this patient population. We considered omitting elevated urinary calcium as an exclusion criterion to boost enrollment, but it would compromise safety and would jeopardize one very important scientific question that is to be answered by this investigation.

Plans to develop a multicenter trial and extend the trial to local oncology practices was also considered. We explored a collaboration with Dr. Mark Pegram at the University of Miami as a large single site collaboration partner in February and March 2011. Unfortunately, the collaboration was not feasible due to insufficient funds and the time required to launch a multicenter trial. It is estimated to take 6-8 months to receive the necessary approvals for a multicenter trial and it would make administration and costs much higher than originally budgeted for. Thus, opening a multicenter trial is beyond the scope of this award.

The Stanford Scientific Review Committee audited the trial in October 2010 and they voiced concerns regarding low recruitment and planned another review in 6 months with possible closure if recruitment remains low. We continued to screen thousands of office visits at the Stanford Cancer Center and continued to approach many breast cancer patients. Despite all our efforts, the recruitment remained low and the trial had to be

closed. The Stanford IRB closed the trial on April 13, 2011. The final repot was submitted to the FDA and the IND was pulled on April 1, 2011. The trial has been removed from the Stanford Clinical Trials Directory and the status update is currently processed by www.clinicaltrials.gov.

Here are the data submitted to the FDA in the final report:

Results:
Subject recruitment:
Goal: 100 subjects
Screened thousands of clinic encounters at Stanford Cancer Center
Approached over 150 patients
Consented 16 (not eligible 8)
Enrolled 8
Completed 5
Withdrawn: 0

Demographics:
Race and ethnicity:
White, non-Hispanic: 71%
Asian: 29%
Age groups:
50-59 years old: 57%
60-69 years old: 43%

No serious adverse events occurred. No participants dropped out or were lost to follow-up.

Despite the difficulties in enrollment, the trial was recognized by the Stanford Cancer Center by an award for Outstanding Performance in Clinical Research on April 26, 2011. Please see that award attached in Appendix 1.

Task 5. Final clinical data analysis and preparation for publication (months 34-36)

a. Clinical data analysis with help from epidemiologist mentor and statistician
b. Manuscript preparation

This task is not been completed due to closure of the clinical study because of low recruitment. Data are not sufficient to perform analysis and to generate publications.

Task 6. Trainee attends Clinical Research Training Program (months 1-24)

a. Core courses in biostatistics, epidemiologic methods, clinical trials, data management and research ethics
b. Master thesis preparation
c. Master of Science (MS) degree in Clinical Epidemiology

This task has been completed. The recipient completed studies and graduated with a Masters in Clinical Epidemiology degree in April 2010.

Task 7. Manuscript preparation on the optimal dosing of vitamin D for subjects with breast cancer (months 24-48)

This task has not been completed due to closing of the clinical trial. The recipient wrote a manuscript of an opinion paper regarding the optimal dosing of vitamin D in breast cancer patients (this is part of the thesis work, please see in Appendix 3). Unfortunately, due to closure of the clinical trial and lack of supporting data on the optimal dose of vitamin D for breast cancer patients, the paper is too weak to be considered for publication.

Key Research Accomplishments:

Unfortunately, there are no sufficient research findings to generate original publications. Despite all our efforts, we were not able to collect meaningful amounts of data from the clinical trial. However, we learned that this scientific question needs to be addressed in a much larger multicenter trial which is outside of the cope of this postdoctoral award. Important learnings from this clinical trial can be used to design a larger multicenter clinical trial to investigate the role of vitamin D in preventing aromatase inhibitor-induced bone loss in women with breast cancer. These important findings include:

1. the need for a larger, multicenter trial to evaluate the vitamin D status of women with breast cancer and to evaluate the role of optimizing vitamin D status in preventing aromatase inhibitor-induced bone loss
2. larger than previously expected proportion of women entering aromatase inhibitor therapy already have biochemical signs of bone loss despite normal bone mineral density. These women are potentially more vulnerable to the negative side effects of aromatase inhibitors and need to be followed carefully.
3. It is surprising how many breast cancer patients were not willing to be randomized to different vitamin D doses. An open label prospective study could be designed to follow these subjects and monitor their vitamin D status and bone markers.
4. Vitamin D appears to be safe and well tolerated in our small cohort, as no adverse events occurred. However, our cohort remained too small to conclude that 2400 IU vitamin D is safe for women with breast cancer.

Another important accomplishment is the positive impact this career development award made on the recipient. Persistence in the face of challenges and failures helped the recipient to become more resilient and innovative. The recipient made the transition to a successful translational investigator. The award is terminated early because the recipient accepted a medical director position at a major medical group. Thus, the knowledge the recipient accumulated at Stanford University will be used to improve health care delivery and medical outcomes on a large scale, for breast cancer patients and far beyond.

Reportable Outcomes:

Due to difficulties with respect of data collection from the clinical trial and the outcomes study, there are no original publications to report.

Poster presentation:
Balint E, Carlson RW, Whittemore AS, Karpf DB: The role of vitamin D in aromatase inhibitor-induced bone loss. Leading Innovation and Knowledge Sharing (LINKS) meeting of the Department of Defense Breast Cancer Research Program, Chantilly, VA, February 16-17, 2011.
Attached please find the poster in appendix 2.

Degree: Masters in Clinical Epidemiology, Stanford University, Stanford, CA
Please see Thesis attached in appendix 3.

Employment: Medical Director, Brown and Toland Medical Group, San Francisco, CA

Conclusions:

During the reporting period several tasks have been completed and others could not be completed due to failure to collect sufficient data from the clinical trial and early termination of the award. Despite our efforts, the clinical trial had to be closed due to low recruitment. No serious adverse events occurred. Recruitment remained low due to geography of patients, patients not willing to be randomized, clinical trial fatigue and subjects screening out due to high urinary calcium excretion and/or osteoporosis. Expanding the study into a muticenter clinical trial was not feasible due to financial and time constrains, thus a muticenter clinical trial is beyond the scope of this award. Our work was recognized by the Stanford Cancer Center by an award for Outstanding Performance in Clinical Research. The recipient completed her studies and graduated with a degree of Masters of Science in Clinical Epidemiology. The SEER-Medicare outcomes analysis was not completed due to early termination of the award.
The award provided insights into the field of bone disease in breast cancer patients that can be used when designing clinical trials in the future. The award resulted in employment for the recipient as a medical director thus the knowledge will be used on a large scale to improve outcomes for patients with breast cancer and other conditions.

References:

N/A

Appendices:

Appendix 1. Award: Outstanding Performance in Clinical Research from the Stanford Cancer Center, April 26, 2011

Appendix 2: Poster
Balint E, Carlson RW, Whittemore AS, Karpf DB: The role of vitamin D in aromatase inhibitor-induced bone loss. Leading Innovation and Knowledge Sharing (LINKS) meeting of the Department of Defense Breast Cancer Research Program, Chantilly, VA, February 16-17, 2011.

Appendix 3: Thesis: Vitamin D, Breast Cancer and Bone Health
Submitted to the Division of Epidemiology, Department of Health Research and Policy, Stanford University School of Medicine

Stanford Cancer Institute

For Outstanding Performance in Clinical Research

This Honor is Bestowed Upon

Principal Investigator: Eva Balint, MD, MS

Study Coordinator: Charlene Kranz, BS

Beverly S. Mitchell

Beverly S. Mitchell, MD

April 26, 2011

The role of vitamin D in aromatase inhibitor-induced bone loss

Balint E, Carlson RW, Whittemore AS, Karpf DB

Departments of Medicine and Oncology and Health Research and Policy, Stanford University, Stanford, California

Introduction

Aromatase inhibitors (AIs) effectively suppress breast cancer growth and increasingly used in the adjuvant setting to prolong disease-free survival. Their effect is mediated by profound suppression of circulating estrogen (up to 98% postmenopausal women) [Lonning 2008]. AI use is limited by their severe side effects including muscle and joint pains as well as bone loss leading to a substantially increased risk of fractures. Using the AI anastrozole for 2 years, bone mineral density (BMD) was reduced about 4% in the spine and hip [Dowsett 2005]. Currently no therapy is available to ameliorate these side effects. Bone loss occurs in all women receiving AI's, and about 50% of patients with initial normal BMD become osteopenic while receiving AI therapy, secondary to local estrogen deficiency in the bone [Dowsett 2005]. Daily doses of 400-1000 IU vitamin D are currently recommended for optimal bone health. Vitamin D administration improves balance and muscle function and reduces fall risk in the elderly [Bischoff-Ferrari 2004]. We hypothesize that vitamin D deficiency might play a role in bone loss and muscle pains associated with AI therapy, and vitamin D administration might be effective to treat these symptoms. The Food and Nutrition Board selected the daily dose of 2400 IU vitamin D as the no-observed-adverse-effect level (NOAEL) [Food and Nutrition Board 1997]. However, the highest chronic dose for vitamin D intake that cause no adverse effect on adults has not been established, and the optimal daily vitamin D dose has been extensively debated [Hathcock 2007]. There are no clinical data available on NOAEL doses of vitamin D specifically in women with BCa, although there are no reasons to expect differential sensitivity in this sub-population. We hypothesize that 2400 IU vitamin D daily will be well-tolerated in women with breast cancer, and that this dose of vitamin D is potentially effective to treat AI-induced bone loss (thereby reducing fracture risk), as well as muscle and joint pains. Thus, the purpose of the study is to investigate the effect of NOAEL doses of vitamin D for treatment of AI-induced bone loss and muscle pains in patients receiving AIs for breast cancer.

Aromatase inhibitors

Generation	Steroidal (type1)	Nonsteroidal (type 2)
First (non-selective)	—	Aminoglutethimide
Second (selective)	Formestane	Fadrozol
Third (super-selective)	Examestane (Aromasin)	Anastrozole (Arimidex) Letrozole (Femara)

Education/Training

Master of Science (MS) degree in Clinical Epidemiology at Stanford University, working toward a career in translational research in breast cancer.

Clinical trial

Title: *A Phase I/II randomized, double-blind, controlled study to evaluate efficacy and safety of vitamin D on bone mineral density and markers of bone resorption in aromatase inhibitor-induced bone loss in women with breast cancer.*

Study design: Women with breast cancer, who completed primary surgical and/or chemotherapy and who are candidates for adjuvant aromatase inhibitor therapy will be enrolled and randomized to interventional and control groups. The interventional group will be treated with NOAEL dose vitamin D (2400 IU daily), while standard of care vitamin D therapy (800 IU daily) will be administered in the control group. Safety will be monitored every 4 months, while efficacy will be determined after 1 year of treatment.

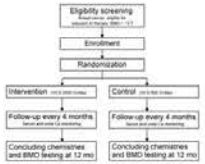
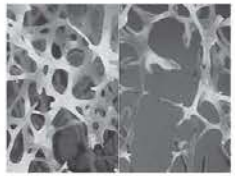


Figure 1: Schematic diagram of study design.

Primary endpoint: spine BMD T-score change over one year.
Secondary endpoints: change in hip BMD T-score, bone turnover markers (NTx, bone-specific alkaline phosphatase), arthralgias and myalgias, serum calcium and fasting spot urine calcium/creatinine ratio.

Sample size by treatment group: 50 subjects per group

Eligibility criteria: Post-menopausal women with histology-confirmed estrogen receptor positive invasive primary breast cancer, who completed primary surgical and chemotherapy and who are candidates for adjuvant therapy with an aromatase inhibitor. Subjects with metastatic disease, elevated urine spot calcium/creatinine ratio, osteoporosis and those taking bisphosphonates are excluded.



Normal bone Osteoporotic bone

Statistical considerations: Analysis will be based on the intent-to-treat population. Complete case analysis will be performed. The study is powered to evaluate efficacy of vitamin D on AI-induced bone loss, as change in spine BMD T-score compared to baseline. Secondary end points will be correlated with vitamin D doses and 25(OH)-vitamin D serum levels using mixed effects analysis.

Web site:
http://med.stanford.edu/clinicaltrials/detail.do?studyId=1302
http://clinicaltrials.gov/ct2/show/NCT00904423

Results:

Subject recruitment

Screened thousands of clinic encounters at Stanford Cancer Center
Approached over 150 patients

Subjects enrolled:	12 (4 screen failures)
Completed:	4
Withdrawn:	0

Demographics

Race, and ethnicity:	White, non-Hispanic 71%, Asian 29%
Age groups:	50-59 years old: 57.2% 60-69 year old: 42.8%

Adverse events: No death or adverse events occurred, none of the subjects withdrew due to adverse events.

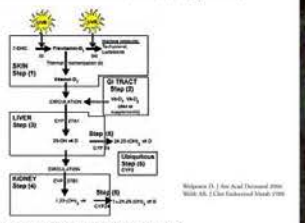
Reasons for low recruitment:
Non-eligible for enrollment (osteoporosis, high urine calcium)
Geographical distance/time to travel for screening
Wanting to take more or less vitamin D than offered in the trial
Not willing to be randomized
Clinical trial fatigue of subjects
Investigator fatigue

Conclusions: This is a clinical trial investigating the effect of NOAEL dose of vitamin D for the prevention of bone loss associated with aromatase inhibitor use for breast cancer in the adjuvant setting. This study is currently recruiting and no results are available yet.

Future directions: Study is too small for multicenter trial.
Intensified recruitment effort at Stanford Cancer Center.

Publication

Balint E, Whittemore A: What dose of supplemental vitamin D should be recommended for women with breast cancer? (review) (in preparation)



Vitamin D synthesis in humans

Outcomes study

Rationale:
Breast cancer is the most common cancer among women. We propose to evaluate the outcomes associated with aromatase inhibitor use outside of a study population. Large interventional trials showed that aromatase inhibitors reduce breast cancer recurrence by about 40%, compared to tamoxifen. We plan to examine the effectiveness of aromatase inhibitor therapy on early ER+ breast cancer, compared to tamoxifen, using the SEER-Medicare database, and compared it to the effectiveness reported in large clinical trials.
Current large prospective trials of aromatase inhibitors reported a wide range of significant bone loss (10-50%). ASCO currently recommends yearly bone mineral density (BMD) testing for women high risk for osteoporosis secondary to aromatase inhibitor, but yearly BMD testing is rarely performed. We plan to ascertain the proportion of women on aromatase inhibitors monitored for bone loss (i.e. undergoing annual BMD testing) as recommended by ASCO.
Recent reports suggest that up to 75% of women with breast cancer also have vitamin D deficiency. Using the SEER-Medicare database, we plan to determine the proportion of breast cancer patients tested and/or treated for vitamin D deficiency.

Aims of the study:
1. The effectiveness of aromatase inhibitor use in preventing breast cancer recurrence, compared to tamoxifen, in a setting outside of clinical trials.
2. Whether women taking aromatase inhibitors receive yearly BMD testing as recommended by ASCO (quality control).
3. Determine the proportion of AI-treated women who have had their vitamin D serum level tested and corrected
4. Determine the incidence of hip fractures, loss of mobility (nursing home care) and death associated with aromatase inhibitor use in the community, compared to tamoxifen.

Materials and methods:
Using the SEER-Medicare database, we will identify women with the diagnosis of breast cancer. We will determine the subset taking aromatase inhibitors using Medicare claims. We will follow these subjects 1995 to 2009 and determine survival, receiving yearly bone mineral density testing, diagnosed and/or treated vitamin D deficiency. We will also determine the rate of hospitalization for hip fracture or death while receiving aromatase inhibitor therapy, compared to tamoxifen.

Results:
This study is currently underway and there are no results available yet.

Acknowledgments

Funding for this project was provided by the Department of Defense Breast Cancer Research Program, U.S. Army Medical Research and Materiel Command, Fort Detrick, MD 21702-5012

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BC062923

For further information

Please contact ebalint@stanford.edu for more information on this project.

Vitamin D, Breast Cancer and Bone Health

A Thesis
Submitted to the Division of Epidemiology
Department of Health Research and Policy
In Partial Fulfillment of the Requirements
For the Master of Science in Epidemiology Degree
Stanford University School of Medicine

Eva Balint, MD
March 2010

Vitamin D, Breast Cancer and Bone Health

Eva Balint, MD
March 2010

Approved for Submission to the Division of Epidemiology
Department of Health Research and Policy
Stanford University School of Medicine

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Department of Health Research and Policy

Co-Reader Reader:
Date:_____

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Philip Lavori, PhD
Professor
Department of Health Research and Policy

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WHAT DOSE OF SUPPLEMENTAL VITAMIN D SHOULD BE RECOMMENDED FOR WOMEN WITH BREAST CANCER? (review)

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Part 2:

VITAMIN D, BREAST CANCER AND BONE HEALTH: A CLINICAL TRIAL

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I am also thankful for Charlene Kranz, whose tireless dedication and effort is indispensable in running the clinical trial, and the collaborator oncologists at Stanford Cancer Center, Alice Guardino, MD, Allison Kurian, MD, Joseph Mollick, MD, Frank Stockdale, MD, Melinda Telli, MD, and Shruti Seth, MD. I appreciate all I learned about vitamin D and bone from David Feldman, MD and from David Karpf, MD. I am thankful for Department of Defense, Breast Cancer Research Program for financial support of the Master's training and the clinical trial, especially my grant managers Dr. Allison Milutinovich, Dr. Hin Lee and Dr. Melissa Green, as well as Paula Glauber from the Human Research Protection Office (HRPO) for their support throughout the seemingly unending obstacles this project encountered through its execution. I would like to express my gratitude to the Endocrine Division, especially Benita Kaeding and Rick Kraemer, MD for their dedication to my success.

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Introduction to the thesis:

This thesis is divided into two parts. The first part provides an overview of the breast cancer/vitamin D field in the form of a review/opinion paper, pointing out gaps in knowledge and providing recommendations for optimizing vitamin D status in breast cancer patients. The second part describes a clinical trial in progress aiming to address the safety and efficacy of vitamin D for prevention of aromatase inhibitor-induced bone loss in breast cancer.

Part 1

Author: Eva Balint, MD

Title: WHAT DOSE OF SUPPLEMENTAL VITAMIN D SHOULD BE RECOMMENDED FOR WOMEN WITH BREAST CANCER? (review)

Abstract:

Background: Vitamin D deficiency and insufficiency are common among women with breast cancer and are currently under-diagnosed and under-treated. Accumulating evidence suggests that optimal vitamin D levels are important not only for bone protection but also for reducing breast cancer incidence and mortality. A healthy lifestyle and/or the currently recommended daily doses of vitamin D (400-800 IU) are only adequate for maintenance of sufficient vitamin D levels but cannot correct vitamin D deficiency. Thus, the currently recommended vitamin D doses are not adequate to meet the needs of most patients with breast cancer. What dose of vitamin D should be recommended for women with breast cancer, for anticancer and bone effects, and what is the optimal way to achieve adequate vitamin D status?

Methods: The literature was reviewed using Pubmed searches with keywords related to vitamin D, breast cancer incidence and survival and bone, such as “vitamin D”, “breast cancer”, “incidence”, “survival”, “bone strength” “bone mineral density” between December 3, 2009 and March 3, 2010.

Results: Evidence accumulates regarding the importance of vitamin D not only for bone health but also for breast cancer outcomes. However, the evidence that vitamin D supplementation reduces breast cancer progression and/or mortality is largely based on observational studies and only a few interventional trials. There is no evidence regarding the effect of vitamin D supplementation on bone loss or fractures in women with breast cancer, but vitamin D supplementation reduces fractures in postmenopausal subjects. Based on the observational studies, it is difficult to determine what dose of supplemental vitamin D is necessary to achieve optimal vitamin D status. While waiting for future more definite evidence, it is important that vitamin D deficiency be identified and treated in breast cancer subjects and those at high risk of breast cancer. Currently recommended vitamin D supplemental doses are only appropriate for patients with normal vitamin D levels. Vitamin D deficient patients require a short course of larger doses of vitamin D to correct deficiency, and a maintenance dose can be continued once the serum 25D levels are in the normal range.

Conclusions: Recognizing and treating vitamin D deficiency in women with breast cancer is of paramount importance. Vitamin D deficiency should be identified and treated at the time of breast cancer diagnosis, and for those at high risk for breast cancer. Currently recommended doses of vitamin D are appropriate for subjects with normal vitamin D levels and for vitamin D deficient subjects after having corrected vitamin D deficiency.

Introduction

Increasing body of evidence suggest that vitamin D deficiency plays an important role in breast cancer incidence, recurrence, mortality as well as bone strength and fractures of patients with breast cancer. Vitamin D deficiency and insufficiency are defined as serum levels of 25-hydroxy-vitamin D (25D) below 50 nmol/L (20 ng/ml) and 50-75 nmol/L (20-30 ng/ml), respectively. Vitamin D deficiency and insufficiency are common among women with breast cancer, and it is currently under-diagnosed and under-treated in this population. It is estimated that as low as about a quarter of subject with breast cancer present with adequate vitamin D status [1-3]. Vitamin D3 (25-hydroxyvitamin D, 25D) is photosynthesized in the human skin from its precursor previtamin D3 via an ultraviolet (UVB) radiation-dependent process. This process is significantly reduced by skin pigmentation and in the ageing skin. Sunscreen is so effective in blocking both UVB radiation and vitamin D production, that it can lead to vitamin D insufficiency even in individuals with significant sun exposure [4]. Air pollution and western lifestyle might be a major contributor to vitamin D deficiency of epidemic proportion due to limited sun exposure even in sunny climates (sunscreen use, clothing, and increasing amount of time spent indoors or on transportation).

Vitamin D and breast cancer prevention

Vitamin D deficiency has been associated with increased incidence and mortality from breast cancer, mostly based on observational and ecologic studies on solar UV radiation exposure [5, 6]. In a recent pooled analysis of two observational studies, subjects in the highest quintile for serum 25D level (52 ng/mL) had a 50% reduction of breast cancer risk, compared to vitamin D deficient subjects (25D below 13 ng/ml) [7]. Based on observational data, the vitamin D-breast cancer connection is controversial. A nested case control study found that cases had lower vitamin 25D levels, compared to controls, and the highest tertile of serum 25D was associated with reduced risk of breast cancer (RR 0.52, 95% CI 0.32-0.85) [8]. Others did not observe a correlation between breast cancer relative risk and serum vitamin D metabolites [9]. In a 4-year randomized, double-blind, placebo controlled trial, 1000 IU vitamin D and calcium supplement was associated with reduced rates of incident cancers (all cancers combined) with a relative risk (RR) of 0.402 (0.20;0.82) in the calcium plus vitamin D arm. Vitamin D treatment assignment and serum 25D levels were both independent predictors of reduced cancer risk observed on the calcium plus vitamin D arm, compared to placebo [10]. Using logistic regression with cancer as outcome and baseline vitamin D as predictor, the authors estimated the RR of 0.983 (0.968;0.997) for cancer per baseline vitamin D unit. Thus, 10 mg/ml increase in serum 25D is associated with a 35%

reduction in cancer risk in this trial [10]. On the other hand, in the Women's Health Initiative (WHI), calcium and a modest amount of vitamin D (400 IU daily), serum vitamin D levels were not associated with breast cancer risk [11] or benign proliferative breast disease, a condition associated with increased breast cancer risk [12]. Of note, relatively small vitamin D doses were administered in the WHI, which might contribute to the lack effect.

Vitamin D and breast cancer mortality and survival

Data are sparse regarding the prognostic effect of vitamin D on breast cancer mortality and survival. Deficient levels of vitamin D are associated with increased breast cancer mortality [13]. Serum vitamin D levels are higher in healthy women compared to women with breast cancer, and women with early breast cancer have significantly higher levels of serum 25D compared to those with locally advanced or metastatic disease [14]. Serum 25D levels were shown to be lower among women with regional breast cancer, compared to those with in situ disease [2]. A prospective inception cohort showed that vitamin D levels at the time of breast cancer diagnosis correlated with long-term distant disease-free survival: vitamin D deficient subjects had an increased risk of recurrence with a hazard ratio of 1.71 (1.02-2.86) [1].

Vitamin D and bone loss and fractures in breast cancer patients

The importance of vitamin D in the etiology and treatment of bone loss cannot be overstated, as vitamin D affects bone physiology and maintenance of bone mass in several important ways [15 16]. Bone health is severely compromised in women with breast cancer, due to estrogen deprivation therapy (chemotherapy and aromatase inhibitors), direct toxic effect of chemotherapy on bone and vitamin D deficiency. There are no studies available on the effect of vitamin D for bone loss and fracture prevention in breast cancer patients. A Pubmed search using keywords "vitamin D" "breast cancer" and various terms related to bone (such as "fracture", "bone loss" and "bone mineral density") did not produce any articles where vitamin D use was randomized or examined. Bone loss in breast cancer due to estrogen depletion is similar to postmenopausal osteoporosis in many aspects, and lessons learned from postmenopausal osteoporosis could be well applied to bone health in beast cancer. Vitamin D plays a central role in age-related bone loss and it can be ameliorated with adequate vitamin D and calcium supplementation, effective in hip fracture prevention among the elderly [17, 18]. Vitamin D is known to regulate aromatase activity and estrogen synthesis in osteoblasts [19, 20]. Moreover, vitamin D-mediated aromatase expression is regulated in a tissue-specific manner, increasing aromatase activity and estrogen synthesis in bone cells and suppressing it in breast cancer cells [20]. These findings underlines the importance of

optimal vitamin D status for breast cancer patients, and imply that vitamin D might be a cheap and safe addition to aromatase inhibitor therapy, to protect bone and potentially reduce breast cancer growth in estrogen receptor positive breast cancer.

Based on the evidence, it appears that adequate vitamin D status is associated with better outcomes in terms of breast cancer incidence and mortality, and will likely lead to improvements in bone strength in breast cancer patients. However, the evidence is largely based on ecological studies and observational studies of serum vitamin D metabolites. While the evidence for vitamin D is fairly strong in the observational studies based on Hill's criteria for causality, it is not clear how the subjects achieved optimal vitamin D status and what dose of vitamin D supplementation was given to the subjects in these studies [21]. Thus, although the evidence points toward benefit of optimizing vitamin D status in breast cancer patients, these studies are difficult to use as a basis to recommend the optimal dose of vitamin D supplementation for breast cancer patients. On the other hand, most of the randomized controlled studies did not show benefits in terms of breast cancer and bone outcomes, most likely due to the small vitamin D doses utilized that are not sufficient to correct underlying vitamin D deficiency and/or insufficiency [3].

What is the best way to achieve adequate vitamin D status in breast cancer patients?

Contrary to popular belief, a well-balanced nutritious diet does not necessarily provide sufficient amounts of vitamin D, and only a few food items are rich in vitamin D (Table 1). Fish oils and fatty fish are the most rich in vitamin D; however consuming large amounts might lead to an overdose of vitamin A. Fortified food items such as milk or orange juice only contain about 100 IU vitamin D per serving, thus not sufficient to maintain adequate vitamin D intake or correct deficiency.

Could food fortification or supplements correct vitamin D deficiency? Unfortunately, most fortified foods contain only small amounts of vitamin D: consumption of 3 glasses of milk provides significant amounts of calcium but not sufficient amounts of vitamin D (Table 1). Also, consumption of fortified foods could not be recommended as a method of correcting vitamin D deficiency or maintaining adequate vitamin D levels, as fortification is prevalent in processed foods and food fortification has been used to market unhealthy food items; about 75% of fortified foods were found to have high fat, sugar or salt content [22]. How about over the counter supplements? Most vitamin preparations contain both calcium and vitamin D, and while they

provide sufficient amounts of calcium, most of their vitamin D content is about 400-800 IU per day, which is not sufficient for vitamin D deficient subjects.

How about increasing sun exposure to take advantage of vitamin D production in the skin? Unfortunately, the UVB spectrum of vitamin D photosynthesis is identical to the spectrum that results in skin cancers, thus prolonged sun exposure for the purposes of treatment or prevention of vitamin D deficiency is not recommended [23]. The vitamin D3 produced in the skin is identical to the nutritional vitamin D3 ingested from foods or vitamin D3 supplements. Thus, vitamin D supplements offer a safer alternative and it remains the preferred approach in maintaining adequate vitamin D status.

Are vitamin D supplements adequate to correct vitamin D deficiency?

Currently recommended daily dose of vitamin D is 400-1000 IU for adults per the Dietary Guidelines for Americans. It is estimated that over 1000 IU vitamin D is needed to correct mild vitamin D insufficiency, and doses over 2000 IU daily are necessary to correct vitamin D deficiency to reach a goal of >32 ng/ml [24]. Although the maximal tolerable dose of vitamin D is not known in humans, daily intake of 2400 IU vitamin D has been designated as the no-observed-adverse-events-level (NOAEL) dose. This dose is considerably higher than the currently recommended daily intake, and safety concerns are one of the major obstacles in recommending this dose.

Is it safe to recommend higher doses of vitamin D?

Major side effect of vitamin D lies in its calcemic potential: large doses of vitamin D will increase serum and urine calcium levels, leading to nausea, vomiting, abdominal pain, and renal stone formation. It is estimated that prolonged daily intake of 10,000 IU vitamin D is necessary to develop symptomatic hypercalcemia [24, 25]. The main concern for renal stone formation is based on the WHI, where 400 IU vitamin D and calcium administration was associated with a small but statistically significant increased relative risk of renal stone formation [26]. This is a very surprising finding, considering the modest calcium and vitamin D intake on the experimental arm (1,000 mg calcium and 400 IU vitamin D daily). It is small but statistically significant change, with questionable clinical significance: the cumulative rate on the calcium and vitamin D arm was 2.47 % (rate of 353 per 100,000 women per year), compared to 2.10 % (rate of 301 per 100,000 women per year) on the placebo arm. The accuracy of the data has been questioned as well, as renal stones were self-reported adverse events and not adjudicated. Increased urinary calcium

excretion is a major risk factor for renal stone formation, and unfortunately, urinary calcium excretion outcome was not collected in the WHI. Paradoxically, restrictions in calcium intake leads to an increase in renal stones in stone formers, via an increase of urinary oxalate excretion, as shown by a 5-year randomized trial [27]. While severe calcium restriction is not beneficial to reduce renal stones, very large amounts of calcium intake can also increase calcium excretion and risk of renal stone formation. Of note, participants in the WHI were allowed to continue their own calcium and vitamin D supplements, leading to estimated calcium intakes of over 2 g/day in some cases, which in itself could lead to increased urinary calcium excretion and renal stone formation. Unfortunately, the subject's own calcium and vitamin D intake in the WHI has not been collected (Marcia Stefanick, personal communication), consequently calcium and vitamin D intake of stone formers cannot be ascertained. Thus, it is difficult to speculate whether the increased renal stone formation reported on the calcium and vitamin D interventional arm was due to calcium, vitamin D, their combination or other factors. Consequently, the WHI's somewhat surprising findings raised serious concerns of calcium and vitamin D safety, but unfortunately the WHI does not have the capacity to substantiate or disprove a causal relationship between calcium and vitamin D supplements and renal stone formation.

Vitamin D is an important regulator of calcium homeostasis and its effect on bone is difficult to separate from the effect of calcium. Based on hip bone mineral density (BDM) in an NHANES population, Bischoff-Ferrari recently showed that high calcium intake is associated with increases in BMD only in women with vitamin D deficiency [28]. For subjects with serum vitamin D levels above 20 ng/ml, no additional benefit is derived from calcium intake above 600 mg/day. Thus, vitamin D status appears to be the dominant predictor over calcium intake, and high calcium intake appears to be critical only for vitamin D deficient subjects. Provided that the vitamin D deficiency is fairly common among women with breast cancer, calcium intake remains an important factor in maintaining bone health. Physicians and the general public are more aware of the importance of adequate calcium intake than the need for vitamin D. Campaigns such as "Got milk?" have been effective in increasing calcium intake but not sufficient for adequate intakes for vitamin D. Moreover, it provides false reassurance of the bone protective effects of calcium intake. It is not uncommon that breast cancer subjects take 1500 mg calcium daily, and only limited amounts of vitamin D (0-400 IU daily). Based on the findings of Dawson-Hughes it appears that calcium intake could be safely reduced to 600 mg daily in subjects with optimal vitamin D levels, without compromising bone health. Thus, considering that large doses of calcium can lead to renal stone formation, it appears to be safer to correct vitamin D deficiency

first and subsequent administration of lower doses of calcium supplementation will suffice to maintain bone health without the increased risk of renal stone formation.

Conclusion and recommendations

Based on the available evidence, vitamin D appears to be crucial for breast cancer patients. Vitamin D is clearly beneficial in optimizing bone health and reducing bone loss associated with severe estrogen deficiency due to chemotherapy and aromatase inhibitor use. Moreover, vitamin D might also be beneficial for reducing incidence, mortality and recurrence of breast cancer, however much more work is required to elucidate this. Clearly, optimizing vitamin D status of breast cancer patients is of paramount importance. Based on the available literature, 2400 IU vitamin D daily appears to be safe, however it is much higher than what is currently recommended by the Food and Nutrition Board. Consequently, clinicians will not be able to recommend it to patients. On the other hand, the currently recommended doses of 400-1000 IU daily clearly not appropriate for women with breast cancer, given that vitamin D deficiency is rampant in this population. What is a physician to do in such a conundrum? In the spirit of *Do No Harm*, we need to explore safe alternatives. Vitamin D deficiency is currently under diagnosed and under treated among women with breast cancer, despite diagnosis is fairly straightforward and safe treatment currently exists for this condition. Thus, awareness needs to be raised to evaluate vitamin D status at the time of breast cancer diagnosis and patients at high risk for breast cancer, and treat vitamin D deficiency, whenever appropriate. Several preparations of vitamin D and its metabolites are currently available. Calcitriol, the active metabolite is produced locally in tissues by local 1-hydroxylase enzyme using nutritional 25D as a substrate, and adequate 25D levels are critical for local calcitriol production and biological effect. Thus, I recommend using nutritional vitamin D (cholecalciferol or ergocalciferol) for patients with breast cancer because of cost as well as it is safer (less calcemic) compared to the active drug, calcitriol.

An example of safely replacing vitamin D is with oral doses of cholecalciferol or ergocalciferol 50,000 IU weekly for 6-8 weeks for patients who are free of conditions that might interfere with vitamin D absorption. The effect of a given cumulative dose appears to be similar, regardless of administration frequency of daily, weekly or monthly administration [29]. Parenteral doses need to be considered for subjects with compromised enteral absorption, however these injections are painful. After a confirmatory test of normalized serum 25D, it is safe and appropriate to continue with the currently recommended maintenance oral dose of 800-1000 IU vitamin D daily. This approach is also in agreement with the current dietary and American Society of Clinical

Oncology (ASCO) guidelines as well. Based on recent studies, calcium intake of about 600 mg/day is sufficient for vitamin D sufficient subjects, which will effectively reduce the risk of renal stone formations due to excessive calcium intake. Studies are underway to evaluate the safety and efficacy of larger than currently recommended doses of vitamin D for bone and cancer-related indications in breast cancer patients.

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Tables:

Table 1: Food items and their vitamin D content (source: Dietary supplements Fact Sheet: Vitamin D, National Institute of Health, USDA Nutrient Database web site) Percent daily value is based on 400 IU daily intake.

Food	**Serving size**	**Vitamin D content (IU)**	**% Daily value**
Cod liver oil	1 Tbs (15 ml)	1,360	340
Salmon, cooked	3.5 oz	360	90
Mackerel cooked	3.5 oz	345	90
Sardines canned in oil, drained	1.75 oz	250	70
Tuna, canned in oil	3 oz	200	50
Eel, cooked	15 oz	200	50
Egg	One whole	20	5
Milk, vitamin D fortified	1 cup	98	25
Orange juice, fortified	1 cup	98	25
Margarine, fortified	1 Tbs	60	15
Pudding prepared with fortified milk	0.5 cup	50	10
Ready-to-eat cereals, fortified	0.75-1 cup	40	10
Liver, beef, cooked	3.5 oz	15	4
Cheese, Swiss	1 oz	12	4
Milk, not fortified	1 cup	10	2.5
Human breast milk	1 cup (250 ml)	3.7	1

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Part 2

VITAMIN D, BREAST CANCER AND BONE HEALTH: A CLINICAL TRIAL

Title:

A Phase I/II randomized, double-blind, controlled study to evaluate efficacy and safety of vitamin D on bone mineral density and markers of bone resorption in aromatase inhibitor-induced bone loss in women with breast cancer.

Abstract:

Background: Aromatase inhibitors are effective in reducing estrogen receptor positive (ER+) breast cancer recurrence, but their use is associated with arthralgias, myalgias, bone loss and fractures. Based on the literature, vitamin D deficiency appears to contribute to the side effects associated with aromatase inhibitor use, and currently recommended doses of vitamin D are not sufficient to reverse vitamin D deficiency. Vitamin D at 2400 IU daily is the no-observed-adverse-effect-level (NOAEL) for vitamin D, but it is considerably higher than the currently recommended doses for adults and patient with breast cancer in the US. This trial investigates the safety and effectivity of NOAEL dose of vitamin D for the prevention of aromatase inhibitor induced bone loss and arthralgias/myalgias.

Methods: This is a randomized, double-blind, controlled prospective trial. Postmenopausal ER+ breast cancer subjects are recruited at the time of starting an aromatase inhibitor in the adjuvant setting. Vitamin D is administered at 2400 IU daily (experimental arm) versus 800 IU daily (control, standard of care). Randomization is stratified on recent SERM use. Primary outcome is change in BMD spine T score, mean change from baseline at 1 year. Secondary endpoints include BMD total hip T score, proportion of subjects with clinically meaningful bone loss, arthralgias and myalgias, changes in markers of bone metabolism, serum vitamin D level and safety endpoints (serum calcium and urinary calcium excretion).

Results: the trial received all necessary approvals and is currently in early phases of recruitment. No interim analysis is planned, and no results are available yet.

Conclusions: Vitamin D at NOAEL dose is expected to be safe and effective for the prevention of bone loss and arthralgias/myalgias associated with aromatase inhibitor use for women with ER+ breast cancer.

Introduction:

Aromatase inhibitors are very effective in the prevention of ER+ breast cancer recurrence, but they cause serious side effects, including bone loss, fractures and muscle and joint pains. These side effects currently limit their use. The purpose of this study is to evaluate the safety and efficacy of vitamin D treatment on aromatase inhibitor-induced bone loss in women with breast cancer.

The vitamin D-cancer field is in dire need of randomized, controlled prospective clinical trials. Lack of well-controlled trials is not entirely surprising, considering that vitamin D is a generic, and funding for large clinical trials for a generic or non-proprietary compound remains elusive. In order to shed light onto the efficacy and safety of vitamin D in preventing aromatase inhibitor-induced bone loss, I designed a randomized, double-blind, controlled prospective clinical trial that will be discussed below. Funding was provided from the Department of Defense Breast Cancer Research Program in the form of a postdoctoral career development award.

This is an ongoing clinical trial at Stanford Cancer Center. Please see the current protocol (version 5) in Appendix A.
Web site at Stanford University:
http://med.stanford.edu/clinicaltrials/publicCancerDisplayDetails.do?studyId=1302

The trial is registered with Clinicaltrials.gov, NCT00904423
http://clinicaltrials.gov/ct2/show/NCT00904423

Trial design:

This is a phase I/II, randomized, double-blind, controlled prospective study of vitamin D on aromatase inhibitor-induced bone loss in breast cancer patients.

The study has been redesigned several times. Considering that the MTD for vitamin D in humans has not been determined, I entertained a dose-titration scheme in earlier versions of the trial design. Since 2400 IU vitamin D is likely to be well tolerated with few immediate side effects, and major toxicity (renal stone formation, hypercalciuria and hypercalcemia) is expected to develop as a late complication after prolonged exposure, it was expected that the dose will be titrated fairly quickly without major limiting toxic effects. As such, the titrations scheme would have not been very beneficial in increasing safety and reducing toxicity, but would have made the

trial execution much more cumbersome. Thus, the dose titration scheme was omitted and a more simple, straightforward design was adopted (Figure 1).

Subjects are identified at the Breast Oncology Clinic at the Stanford Cancer Center. After signing informed consent, blood and urine chemistries, as well as bone mineral density are measured, to determine eligibility. If all the inclusion criteria are met and none of the exclusion criteria are present, subjects are randomized. Stratified blocked randomization with a random block size of 4, 6 and 8 is used to assign subjects to treatment groups. As selective estrogen receptor modulator (SERM) treatment is common in breast cancer and stopping SERMs is associated with accelerated bone loss, thus stratification is based on recent SERM use. Considering that the trial is double-blind, the blocked randomization scheme was generated by Dr Lavori and shared only with the investigational pharmacy; it is not known to the investigators or the subjects. Please see the current version of the protocol in the appendix, section 3.1 and 3.2 for inclusion and exclusion criteria, and section 3.4 for randomization procedures (appendix A).

Intervention:

Subjects receive 2400 IU vitamin D daily on the experimental arm and 800 IU daily on the control arm. Vitamin D is packaged in capsules of identical appearance, containing 800 IU, or 2400 IU per capsule. All capsules are manufactured from the same lot of vitamin D3, by Vital Nutrients (Middletown, CT). Daily dose of 800 is considered standard of care for women with breast cancer who are not osteoporotic and receive aromatase inhibitor therapy in the adjuvant setting. Subjects return for 4-monthly study visits, for serum and urine chemistries. At 12 months, bone mineral testing is repeated, and concluding serum and urine chemistries are performed. Please see section 4 of the protocol for additional details of the treatment plan (appendix A).

In the design phase of the trial, I considered whether vitamin D deficiency be treated, at the time of enrollment. Considering that vitamin D deficiency is under diagnosed and under treated in breast cancer patients, I decided not to correct vitamin D deficiency to reflect the current treatment environment. Moreover, not correcting vitamin D deficiency will enable us to detect whether 2400 IU vitamin D will correct deficiency in subjects over time, and whether toxicity develops. The current trial setup is expected to reflect the hypothetical conditions of a higher than currently recommended vitamin D dose environment for the general population, specifically for breast cancer patients.

Safety:

Calcium excretion is carefully monitored throughout the trial. The WHI reported a small but significant increase in renal stone formation with 400 IU vitamin D compared to placebo [26]. Rates of renal stone formation are surprisingly high in the WHI, and an increase in renal stone formation was not expected with the modest doses of calcium and vitamin D supplements administered in the WHI. Increased urinary calcium excretion is the major risk factor for renal stone formation. Interestingly, urinary calcium excretion with vitamin D has only been reported with very short exposures, but not with chronic administration of vitamin D. Of note, participants in the WHI were allowed to continue their own calcium and vitamin D supplements, potentially leading to calcium intakes over 2 g/day in the experimental group. Thus, it is difficult to speculate whether the increased renal stone formation reported with 400 IU vitamin D in the WHI was due to calcium, vitamin D, their combination or other factors. The current trial is designed to explore whether 2400 IU vitamin D will elevate urinary calcium levels and increases the risk of renal stone formation.

Endpoints:

Primary endpoint is change in BMD spine T score, mean change from baseline at 1 year. Secondary endpoints include BMD total hip T score, proportion of subjects with clinically meaningful bone loss, arthralgias and myalgias, changes in markers of bone metabolism, serum vitamin D level and safety endpoints (serum calcium and urinary calcium excretion). Please see section 12 of the protocol for endpoints and statistical considerations.

Analysis:

Analysis will be performed on the entire randomized study population (intent to treat). Complete case analysis will be performed. We intend to enroll 50 subjects per study arm. The primary outcome (change in spine BMD T-score, continuous outcome) will be analyzed, using analysis of covariance. The null hypothesis is that 2400 IU dose of vitamin D will not result in a significant difference in BMD at one year, compared to controls (800 IU, standard of care). We plan to enroll 100 patients, which powers the study for the efficacy outcome (primary outcome, change in spine BMD T-score). The dropout rate is estimated 15%. A 2.6 % reduction in spine and 1.7% reduction in hip BMD T-score have been reported in patients taking an aromatase inhibitor for 1 year [30, 31]. With 50 subjects on each arm, the study has 80% power to detect a change that is 0.57 times the SD, and 90% power against 65% of SD. This is a medium size effect. Since no precise estimate of the SD is currently available, a rough estimate of about 3.0, based on the ATAC trial,

is used [30]. Based on this SD estimate, a 57% SD change would translate to a 1.6% change in spine BMD. In the ATAC study, anastrozole caused a 2.6% drop, and tamoxifen lead to a 1.2% gain in hip BMD at 1 year, with a net difference of 3.8% between these two groups. Compared to tamoxifen, 2400 IU vitamin D is expected to result in a smaller fraction of change in BMD, but a clinically meaningful reduction in AI-induced bone loss.

This is a small study, and interim analysis is not planned. Safety will be monitored in an on-going basis. The 2400 IU dose of vitamin D is considered safe, thus stopping the study for safety is not planned. Considering that the primary end point (BMD) will be only measured at 1 year for each subject and it is estimated that all subjects will be enrolled by the time efficacy data becomes available for the first 50 subjects, interim analysis will not be performed for efficacy either.

Review process/monitoring:

The study has been reviewed at by the Breast Cancer Disease Management Group, the Scientific Review Committee and the Institutional Review Board at Stanford University; the Institutional Review Board at the Department of Defense (DoD) and the Food and Drug Administration (FDA).

The MTD of vitamin D is humans is not known, and the vitamin D dose used in the experimental arm of this trial is higher than currently recommended for this indication. Thus, the trial is considered a phase I/II and an Investigational New Drug application (IND) is required from the FDA. Considering that this is an investigator-initiated trial, I hold the IND (IND 103547) as the sponsor/investigator of the study.

The study is monitored by the Stanford Cancer Center, a Medical Monitor, the IRB and the DoD Human Research Protection Office (HRPO). Please see section 7.2 (adverse event reporting) in details on the data safety and monitoring plan.

Results:

Subjects are identified at the time of their oncology clinic visits. We screen medical records of all patients visiting the Stanford Cancer Center Oncology Clinics of our oncologist collaborators, Drs. Robert Carlson, MD, Alice Guardino, MD, Allison Kurian, MD, Joseph Mollick, MD, Frank Stockdale, MD, Melinda Telli, MD, and Shruti Seth, MD. Subjects are identified while undergoing diagnostic or therapeutic procedures for their breast cancer (staging, chemotherapy,

radiation). Subjects are consented at the time when they discuss aromatase inhibitor use with their treating oncologist and receive their prescription. If they meet inclusion and none of the exclusion criteria, subjects are randomized within 6 weeks of starting an aromatase inhibitor. We screened over 1200 breast oncology clinic visits, and enrolled 8 subjects so far. Out of these, 3 subjects did not meet inclusion criteria (elevated baseline urine calcium excretion, calcium/creatinine ratio over 0.2). To date, five subjects have been randomized.

Subject recruitment:
Total number of subjects planned: 100
Subjects enrolled: 8 (3 screen failures)
Completed: 0
Withdrawn: 0
Active on study: 5

Demographics:
Race, and ethnicity: White, non-Hispanic 100%
Age groups: 50-59 years old: 57.2%
60-69 year old: 42.8%

Adverse events: No death or adverse events occurred, none of the subjects withdrew due to adverse events.

Conclusions and Future directions:

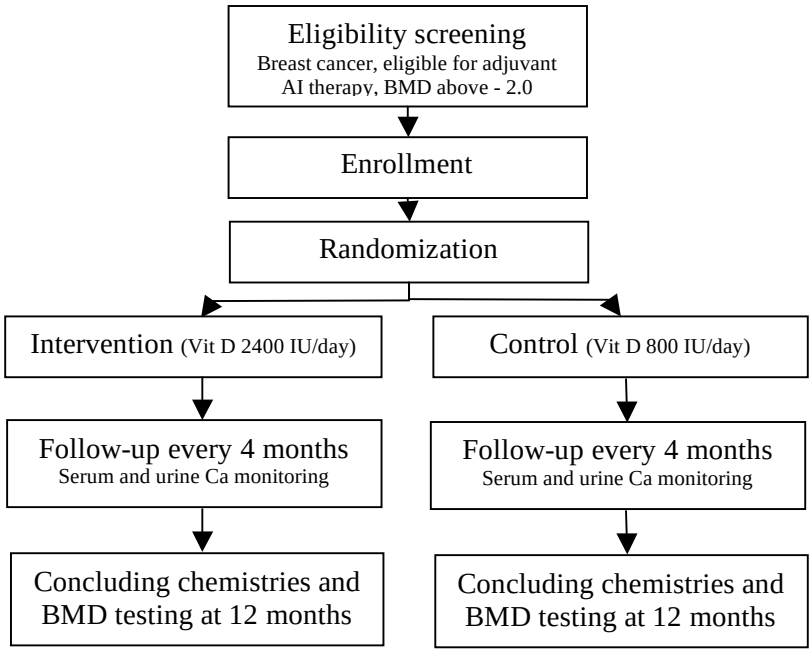
This is a clinical trial investigating the effect of NOAEL dose of vitamin D for the prevention of bone loss associated with aromatase inhibitor use for breast cancer in the adjuvant setting. This study is in the early phase of subject recruitment and no results are available yet. Despite optimizing identification of subjects and recruitment, our recruitment is currently slower than anticipated. The major reason is that we overestimated the number of eligible subjects that will be recruited from the Stanford Cancer Center. We are planning to expand the study to other medical institutions with a breast cancer clinic, and we are currently in the process of identifying institutions and oncologists as possible future collaborators.

Depending on availability of funding, we are also planning to extend the trial to follow breast cancer outcomes beyond the first year (potentially up to 3-5 years). I am currently in the process

of exploring this option and will write a proposal shortly. Differential effect of vitamin D, such a inhibiting breast cancer growth and protecting bone is biologically plausible, thus the extension of trial to include medically meaningful breast cancer outcomes is a feasible proposal, with high likelihood of success.

Figures:

Figure 1: Outline of the trial



Appendix

Appendix A

Current protocol (version 5)

Appendix B

Study pamphlet

A Phase I/II randomized, double-blind, controlled study to evaluate efficacy and safety of vitamin D on bone mineral density and markers of bone resorption in aromatase inhibitor-induced bone loss in women with breast cancer.

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Agent: Vitamin D (IND 103547)

Protocol Original: 9-July-2008
Protocol Version #2: 29-October-2008
Protocol Version #3: 19-December-2008
Protocol Version #4: 17-June-2009
Protocol Version #5: 17-August-2009

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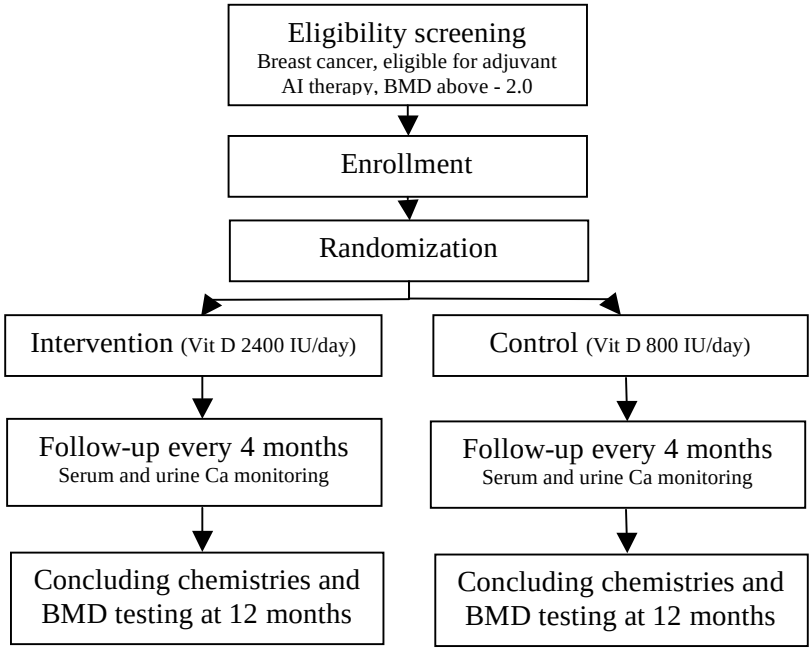
APPENDICES
A. Participant Eligibility Checklist

PROTOCOL SYNOPSIS

TITLE	**A phase I/II randomized, double-blind, controlled study to evaluate efficacy and safety of vitamin D on bone mineral density and markers of bone resorption in aromatase inhibitor-induced bone loss in women with breast cancer.**
STUDY PHASE	**I/II**
INDICATION	**Aromatase-inhibitor induced bone loss in breast cancer**
PRIMARY OBJECTVES	**To evaluate the efficacy of vitamin D treatment on aromatase inhibitor-induced bone loss and myalgias in women with breast cancer.**
SECONDARY OBJECTIVES	**To evaluate the safety of no-observed-adverse-effect-level (NOAEL) doses of vitamin D in women taking aromatase inhibitors for breast cancer.**
HYPOTHESES	**Aromatase inhibitors are potent suppressors of breast cancer growth, but side effects include bone loss, fractures, arthralgias and myalgias. We hypothesize vitamin D administration might be beneficial in treating these symptoms and to protect bone.**
STUDY DESIGN	**Women with breast cancer, who completed primary surgical and/or chemotherapy and who are candidates for adjuvant aromatase inhibitor therapy will be enrolled and randomized to interventional and control groups. The interventional group will be treated with NOAEL dose vitamin D (2400 IU daily), while standard of care vitamin D therapy (800 IU daily) will be administered in the control group. Safety will be monitored every 4 months, while efficacy will be determined after 1 year of treatment.**
PRIMARY ENDPOINTS AND SECONDARY ENDPOINTS	**Primary endpoint is spine BMD T-score change over one year. Secondary end points are: change in hip BMD T-score, bone turnover markers (NTx, bone-specific alkaline phosphatase),**

	arthralgias and myalgias, serum calcium and fasting spot urine calcium/creatinine ratio.
SAMPLE SIZE BY TREATMENT GROUP	**50 subjects per group**
SUMMARY OF SUBJECT ELIGIBILITY CRITERIA	**Post-menopausal women with histology-confirmed invasive primary breast cancer, who completed primary surgical and chemotherapy and who are candidates for adjuvant therapy with an aromatase inhibitor.**
INVESTIGATIONAL PRODUCTS DOSAGE AND ADMINISTRATION	**2400 IU vitamin D per mouth daily in the interventional group.**
CONTROL GROUP	**Standard of care vitamin D therapy: 800 IU per mouth daily.**
PROCEDURES	**Bone mineral density testing at enrollment and conclusion (1 year); serum and urine chemistries every 4 months.**
STATISTICAL CONSIDERATIONS	**Analysis will be based on the intent-to-treat population. Complete case analysis will be performed. The study is powered to evaluate efficacy of vitamin D on AI-induced bone loss, as change in spine BMD T-score compared to baseline. Secondary end points will be correlated with vitamin D doses and 25(OH)-vitamin D serum levels using mixed effects analysis.**


Eligibility screening
Breast cancer, eligible for adjuvant AI therapy, BMD above - 2.0
Enrollment
Randomization
Intervention (Vit D 2400 IU/day)
Control (Vit D 800 IU/day)
Follow-up every 4 months
Serum and urine Ca monitoring
Follow-up every 4 months
Serum and urine Ca monitoring
Concluding chemistries and BMD testing at 12 months
Concluding chemistries and BMD testing at 12 months

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

25(OH)D	25-hydroxy vitamin D
AE	Adverse event
AI	Aromatase inhibitor
BALP	Bone-specific alkaline phosphatase
BCa	Breast cancer
BMD	Bone mineral density
CRF	Case report/Record form
CR	Complete response
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
IRB	Institutional Review Board
LLN	Lower limit of normal
NOAEL	No-observed-adverse-effect-level
NTx	Cross-linked N-telopeptide of type I collagen
PR	Partial response
PTH	Parathyroid hormone
RR	Response rate
SAE	Serious adverse event
SD	Standard deviation
ULN	Upper limit of normal
UNK	Unknown
WHO	World Health Organization
WNL	Within normal limit

1. OBJECTIVES

1.1. Primary Objectives

Aromatase inhibitors (AIs) are very effective and widely used in breast cancer (BCa) in the adjuvant setting to prevent recurrence and prolong disease-free survival, but their use is associated with serious side effects: bone loss, bone fractures, arthralgias and myalgias. The primary objective of this trial is to determine the efficacy of vitamin D on these side effects associated with aromatase inhibitor use. Primary outcome will be spine bone mineral density T score as a measure of efficacy, and outcome measures will also include markers of bone turnover (NTX, bone-specific alkaline phosphatase [BALP]), arthralgias and myalgias, and estimated fracture risk (FRAX score).

1.2. Secondary Objectives

Vitamin D use can be associated with hypercalcemia, hypercalciuria and renal stone formation. The secondary objective is to determine safety of NOAEL dose of vitamin D in women with breast cancer taking aromatase inhibitors. Relevant secondary end points include serum calcium and fasting spot urine calcium/creatinine ratio.

2. BACKGROUND

2.1 Study Disease:

Breast cancer (BCa) is the most common cancer in women. Potent AIs effectively suppress BCa growth and increasingly used in the adjuvant setting to prolong disease-free survival. Their effect is mediated by profound suppression of circulating estrogen (up to 98% postmenopausal women) [Lonning 2008]. AI use is limited by their severe side effects including muscle and joint pains as well as bone loss leading to a substantially increased risk of fractures. A 2.6 % reduction in spine and 1.7% reduction in hip BMD T-score have been reported in patients taking the aromatase inhibitor anastrozole for 1 year, and the T-score reduction is close to 4% at 2 years [Eastell 2006, Dowsett 2005]. Currently no therapy is available to ameliorate these side effects. Currently only women with very low BMD (below -2.0) receive bisphosphonate therapy to prevent further bone loss; others are treated with standard doses of calcium and vitamin D (800-1000 IU/day). However, bone loss occurs in all women receiving AI's, and about 50% of patients with initial normal BMD become osteopenic while receiving AI therapy (clinically important loss of BMD), secondary to local estrogen deficiency in the bone [Dowsett 2005]. Daily doses of 400-1000 IU vitamin D are currently recommended for optimal bone health. Daily doses of 800 IU Vitamin D appears to reduce hip and non-vertebral fractures in elderly persons [Chapuy 1992, Bischoff-Ferrari 2005], however other reports did not confirm these results [Grant 2005, Porthouse 2005]. Vitamin D administration improves balance and muscle function and reduces fall risk in the elderly [Bischoff-Ferrari 2004]. In one report, vitamin D deficiency has been found in 93% of patients presenting with musculoskeletal complaints [Plotnikoff 2003]. We hypothesize that vitamin D deficiency might play a role in bone loss and muscle pains associated with aromatase inhibitor

therapy, and vitamin D administration might be effective to treat these symptoms. Evidence suggests that higher than currently recommended vitamin D doses are necessary for non-skeletal effects and prevention or treatment of cancers. Based on case-control studies, higher blood levels of 25-hydroxy vitamin D is associated with a 50% reduction in breast cancer risk in the highest quartile for vitamin D, compared to the lowest quartile (serum 25OHD level of 52 ng/ml vs 13 ng/ml) [Garland 2007]. To reach this serum level, daily administration of 4000 IU vitamin D is necessary. The Food and Nutrition Board selected the daily dose of 2400 IU vitamin D as the no-observed-adverse-effect level (NOAEL) [Food and Nutrition Board 1997]. However, the highest chronic dose for vitamin D intake that cause no adverse effect on adults has not been established, and the optimal daily vitamin D dose has been extensively debated [Hathcock 2007]. While NOAEL doses of vitamin D appears to be safe, there are no clinical data available on NOAEL doses of vitamin D specifically in women with BCa, although there are no reasons to expect differential sensitivity in this sub-population. As an alternative to taking 4000 IU vitamin D daily, it is estimated that daily intake of 2400 IU with very moderate exposure to sunlight is sufficient to reach comparable blood levels. We hypothesize that 2400 IU vitamin D daily will be well-tolerated in women with BCa, and that this dose of vitamin D is potentially effective to treat AI-induced bone loss (thereby reducing fracture risk), as well as muscle and joint pains. Thus, the purpose of the study is to investigate the effect of NOAEL doses of vitamin D for treatment of AI-induced bone loss and muscle pains in patients receiving AIs for BCa.

2.2 **Investigational Agent**

In humans, vitamin D3 is synthesized in the sun-exposed skin, from its precursor 7-dehydrocholesterol via photoconversion, or obtained from dietary sources or supplements. The active form of vitamin D3, calcitriol, is produced in the human body by subsequent hydroxylations in the liver and kidney, respectively. A small amount of calcitriol is also produced at the tissue level by local hydroxylation, and this appears to be of paramount importance in BCa as well as in bone and muscle. Similar to other steroid hormones, calcitriol binds to nuclear receptors in target tissues and regulates gene expression. It is hypothesized that the concentration of substrate (vitamin D3) is very important in local calcitriol production. Emerging evidence suggests that administration of vitamin D, as opposed to calcitiol, appears to be safer and more effective in modulating target tissue effects in humans.

The currently recommended maintenance vitamin D dose for adults is 400-1000 IU cholecalciferol (vitamin D3) daily. Evidence indicates that higher than currently recommended doses are necessary for effects in cancer. Thus, the current dose recommendations are heavily debated and expected to increase in the near future. According to the Food and Nutrition Board, daily administration of 2400 IU is NOAEL for vitamin D and it is considered safe without significant adverse events [Hathcock 2007].

Major side effects of vitamin D are related to hypercalcemia, and clinical manifestations include polydipsia, polyuria, nausa, vomiting, abdominal pain, pancreatitis, headache, irritability, somnolence, and renal stone formation. Vitamin D toxicity will occur at serum 25(OH)D levels above 100 ng/ml (250 nmol/L), which would require a continuing oral intake in excess of 10,000 IU daily [Heaney 2005]. High doses of vitamin D may lead to hypercalcemia, hypercalciuria and increased risk of renal stone formation, however it is unclear what dose of vitamin D would correspond to an elevated risk of renal stone formation. Higher than currently recommended doses of vitamin D (up to 4,000 IU daily) have been administered to humans without elevation of serum or urine calcium or serious side effects [Hathcock 2007]. On the other hand, the Women's Health Initiative reported a higher than expected incidence of kidney stone formation in the treatment group with 400 IU vitamin D and concurrent calcium intake, in addition to estrogen. Unfortunately, the study design allowed participants to take their own (unknown amounts) of calcium and vitamin D supplements in addition to the study drug, thus it is unclear how much calcium and vitamin D exposure occurred in the experimental group. It is estimated that subjects in the experimental group might have consumed relatively high doses of calcium, potentially well above 2,000 mg daily with over 1200 IU vitamin D. Although it is suspected that renal stone formation was related to high calcium intake in the WHI, it is not possible to clarify whether it was indeed related to high doses of calcium, vitamin D, estrogen or other factors [Jackson 2006]. Based on the vitamin D literature, much higher than NOAEL vitamin D doses have been administered without observing elevations in serum calcium or kidney stone formation. Elevated urine calcium levels are a sensitive indicator of increased risk of renal stone formation. Thus, in the current study, to avoid increased risk of renal stone formation, we will 1) use safe NOAEL doses of vitamin D; 2) will avoid excessive calcium intake; and 3) advise patients to remain well hydrated and will carefully monitor urinary calcium excretion. Although considered safe, daily doses of 2400 IU vitamin D is much higher than the currently recommended daily intake, thus we will monitor subjects every 4 months for potential side effects, including hypercalcemia and hypercalciuria.

2.3 **Rationale**

Accumulating evidence indicates that higher than currently recommended doses of vitamin D are effective in the prevention and treatment of cancers. AI administration is associated with increased bone loss, fractures as well as joint and muscle pains, which often leads to discontinuation of therapy in patients not able to tolerate these side effects. Currently no treatment is available to alleviate these side effects. We hypothesize that co-administration of vitamin D at 2400 IU daily along with AIs is safe and will be effective to treat AI-induced bone loss, as well as AI-induced muscle and joint pains. If this dose of vitamin D is proven safe and effective in women with BCa, it will open new avenues to investigate the role of high dose vitamin D in prevention of AI-induced fractures and the prevention and/or treatment of BCa in the future.

2.4 **Correlative Studies Background**

There are no correlative studies included in this protocol.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

3.1.1 All postmenopausal women with histology-confirmed invasive primary breast cancer, who have completed primary therapy (surgical or XRT with or without adjuvant chemotherapy) and are candidates to receive adjuvant therapy with aromatase inhibitors will be screened for eligibility. Subjects undergoing XRT while taking aromatase inhibitors are eligible. Postmenopausal is defined as satisfying one or more of the following criteria: having had bilateral oophorectomy; aged more than 60 years; or aged 55-59 years with an intact uterus and amenorrheic for at least 12 months; or if amenorrheic for more than 12 months (after receiving hysterectomy, hormone therapy or chemotherapy).

3.1.2 At the time of study enrollment, participants will have completed primary surgical therapy with or without adjuvant chemotherapy. Subjects may undergo XRT while enrolled in the study and taking aromatase inhibitors. Participants will take aromatase inhibitors, having started no more that 6 weeks prior to enrollment in the study.

3.1.4 Participants will be women between 18-85 years of age. Women and minorities will be actively recruited. Though breast cancer extremely rarely occurs in children and men, this study will only recruit adult females.

3.1.5 Participants will have a life expectancy of at least 5 years.

3.1.6 Participants will have ECOG (Eastern Clinical Oncology Group) performance status 0-2.

3.1.7 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Medications affecting bone metabolism (bisphosphonates, anticonvulsants, chronic heparin therapy, chronic glucocorticoid use > 5 mg/day prednisone or equivalent, teriparatide).

3.2.2 Metastatic breast cancer.
High risk for osteoporosis/fractures (BMD < -2.0, history of non-traumatic fracture).
Active hyperparathyroidism
Hypercalcemia
Hypercalciuria (fasting spot urine calcium/creatinine ratio >0.20)
History of renal stones
Diagnosis of stage 3 or higher chronic renal insufficiency or creatinine outside the normal range.
Inability to absorb vitamin D due to intestinal conditions

3.2.3 Considering that vitamin D3 is produced by the human body, allergy to vitamin D3 is not expected to develop. Subjects with known history of allergic reaction to compounds used to manufacture capsules (rice powder) will be excluded form this study.

3.2.4 Recent history of excessive alcohol or drug use. Excessive alcohol use (3 or more servings per day) is associated with bone loss (FRAX WHO Fracture Risk Assessment online tool http://www.shef.ac.uk/FRAX/index.htm).

3.2.5 As this study will recruit post-menopausal patients, thus pregnant or nursing patients are not part of this investigation.

3.2.6 This study is designed to study women after completing primary therapy for breast cancer. Survivors of previous cancers and HIV-positive subjects will not be excluded.

3.3 Informed Consent Process

Subjects with BCa will be contacted by the study coordinator or the Principal Investigator (PI) by telephone and, if the subject is interested, a consent form will be sent to the patient. The protocol will be explained in detail to the subject, including risks and benefits. Subjects will be given the opportunity to ask questions regarding the protocol. Informed consent will be obtained prior to enrollment to the study. The PI will determine whether the subjects have the capacity to consent. If a subject lacks such capacity due to cognitive impairment, age or other causes, the subject will not be recruited. Consent will not be obtained from a legally authorized representative. Translator services will be utilized as needed for non-English speaking subjects.

3.4 Randomization Procedures

Stratified blocked randomization with a random block size of 4, 6 and 8 will be used to assign subjects to treatment groups. As selective estrogen receptor modulator (SERM) treatment is common in breast cancer and stopping SERMs is associated with accelerated bone loss, thus stratification will be based on concurrent or recent SERM use. Treatment blocks will be generated by the biostatistician and communicated to the pharmacy staff, which will mail the appropriate treatment to the participants. This is a double-blind study, and the PD and the research staff will be blinded as well. Subjects will be blinded to the experimental assignment, as vitamin D will be dispensed in identical appearing capsules and all interventions, including the monitoring every 4 months will be identical.

4. TREATMENT PLAN

4.1 Investigational Agent or Device Administration

Eligibility screening: All postmenopausal women with histology-confirmed invasive primary breast cancer, who have completed primary surgical or XRT with or without adjuvant chemotherapy and are candidates to receive adjuvant therapy with aromatase inhibitors will be screened for eligibility. Subjects who meet all inclusion criteria and

none of the exclusion criteria will be included. Subjects with spine or total hip T-scores on DXA < -2.0 and those taking bisphosphonates, anticonvulsants, heparin or teriparatide will not be eligible for the study.
Subjects will undergo an initial clinical laboratory assessment: serum calcium, phosphorus, creatinine, albumin, 25(OH)D, parathyroid hormone (PTH), bone-specific alkaline phosphatase and urine spot calcium/creatinine ratio (fasting) and N-telopeptide (NTx) (second morning specimen). A total of 20 ml's (4 teaspoons) blood will be drawn. Bone mineral density testing (BMD) by dual energy x-ray densitometry (DXA) at the start of adjuvant AI therapy is standard of care. If bone mineral density testing has been done more than 4 months prior to enrollment, it will be repeated for baseline value and cost covered by the study (no charge to the participant). Fracture risk information will be collected using the FRAX questionnaire, and fracture risk will be calculated using the FRAX WHO Fracture Risk Assessment online tool http://www.shef.ac.uk/FRAX/index.htm Subjects will be scheduled to start the study no sooner than 1 day and no more than 30 days after eligibility screening.

Subjects in the control group will receive 800 IU vitamin D3 daily for the entire study period, which is the currently recommended intake and standard of care. Subjects in the intervention group will receive 2400 IU per day. According to the Food and Nutrition Board, daily administration of 2400 IU is the NOAEL for vitamin D and it is considered safe without significant adverse events [Hathcock 2007].

Every 4 months, subjects will return for an assessment. Joint and muscle pains as well as other adverse events will be recorded. Blood will be collected for serum calcium, albumin, and urine calcium and creatinine will be also measured (fasting). At the 4-month assessment, 25(OH)D, bone-specific alkaline phosphatase and urine NTX (second morning specimen) will be measured as well. A total of 20 ml's (4 teaspoons) blood will be drawn at 4, 8 and 12-month assessment.

Vitamin D toxicity will be monitored every 4 months using albumin-corrected serum calcium levels. Spot fasting urine calcium/creatinine ratio will be monitored as well. Subject's dietary calcium intake will be estimated during study visits using a calcium intake assessment checklist, and total calcium intake (dietary plus supplements) will be adjusted to aim for a total of 1000 mg/day. Considering that dehydration and high dietary calcium intake can cause elevated urinary calcium levels while serum calcium remains normal, subjects with elevated spot fasting urine calcium/creatinine ratio above 0.20 and normal serum calcium levels will be instructed to limit total calcium intake to no more than 1000 mg/day and to increase fluid consumption to 64 oz per day.

At 12 months, subjects will undergo a final clinical laboratory assessment: serum calcium, creatinine, albumin, 25(OH)D, bone-specific alkaline phosphatase and fasting spot urine calcium/cretinine ratio and urine NTx (second morning specimen). A total of 20 ml's (4 teaspoons) of blood will be drawn. Morning stiffness, myalgia and joint pain as well as other adverse events will be recorded. BMD will be repeated.

Tissue or blood samples will not be retained or used for future research.

The procedure for modifications to this protocol will include approval of the local IRB as well as Human Subjects Research Review Board (HSRRB) prior to implementation. "The procedure for any modifications to this protocol will include approval of the local IRB. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects will be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance."

"The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO."

"A copy of the approved continuing review report and the local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available."

"The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO."

4.2 General Concomitant Medication and Supportive Care Guidelines

In addition to the study drug (vitamin D), patients will receive standard of care treatment for BCa, which will include aromatase inhibitors. Subjects will have their bone mineral density measured at the time of enrollment, which is standard of care, as well as at the conclusion of the study as part of this protocol. Monitoring every 4 months with blood draws and urine.

Risks:
Blood draws: risk of bruising, discomfort, and a slight risk of infection and clotting.
BMD testing: usually no complications. There is a small amount of radiation exposure, less than 1/10 of the dose of a standard chest x-ray, corresponds to approximately one day of natural background radiation. As with any diagnostic procedure, there is a risk of uncovering a previously unknown medical condition of the subject

4.3 **Duration of Therapy**

Vitamin D administration will continue up to 1 year.

4.4 **Duration of Follow Up**

As part of this protocol, participants will not be followed after the conclusion of this study. However, they will continue to receive oncology follow-up as indicated for the management of their BCa (standard of care).

4.5 **Criteria for Removal from Study**

A study subject may end participation in this study at any time.
Safety (serum calcium and adverse events, symptoms of hyperalcemia) will be monitored every 4 months. Primary outcome will be only determined at 1 year. Subjects with severe symptomatic hypercalcemia will stop taking vitamin D and calcium supplements, and will be withdrawn from the study.

4.6 **Alternatives**

Subjects will receive adjuvant AI therapy as well as supplemental vitamin D (800 IU daily) which is standard of care, regardless whether they participate in this trial or not. Vitamin D will be administered with the hope to treat AI-induced bone loss and myalgias. Currently there is no known therapy available to prevent AI-induced bone loss in women with BMD above -2.0 or to treat arthralgias and myalgias associated with AI therapy, thus no alternative procedures are available. Therefore, the only alternative for the patient is not to participate in the study at all. No attempt at coercion will be made. To minimize the risk of coercion, the treating oncologists, Dr. Robert W. Carlson, Dr. Alice Guardino, Dr. Allison Kurian,Dr. See Phan and Dr. Melinda Telli will not administer informed consent. Also, once enrolled, subjects are free to withdraw from the study at any time.

4.7 **Compensation**

Except for receiving study drug free of charge and reimbursement for parking, study subjects will not be paid for their participation in this study.

5. INVESTIGATIONAL AGENT PROCEDURE INFORMATION

5.1 **Investigational Agent Procedure**

In humans, vitamin D3 is synthesized in the sun-exposed skin, from its precursor 7-dehydrocholesterol via photoconversion, or obtained from dietary sources or supplements. The active form of vitamin D3, calcitriol, is produced in the human body by subsequent hydroxylations in the liver and kidney, respectively. A small amount of calcitriol is also produced at the tissue level by local hydroxylation, and this appears to be of paramount importance in BCa as well as in bone and muscle. Similar to other steroid hormones, calcitriol binds to nuclear receptors in target tissues and regulates gene expression. It is hypothesized that the concentration of substrate (vitamin D3) is very important in local calcitriol production. Administration of vitamin D, as opposed to calcitriol, appears to be safer and more effective in modulating target tissue effects in humans.

The efficiency of vitamin D photoproduction declines with age [Holick 2005]. Thus, the elderly, and individuals with limited sun exposure are at risk of developing vitamin D insufficiency, requiring oral vitamin D supplementation. After an oral dose of vitamin D3, blood levels begin to rise at 4 hours, peak by 12 hours, and return to close to baseline by 72 hours (half-life 14 hours). Metabolism is primarily via hydroxylation in the liver and kidneys and minimal glucuronidation, excretion via metabolites in urine (2.4%) and feces (4.9%).
The currently recommended maintenance vitamin D dose for adults is 400-1000 IU vitamin D3 (cholecalciferol) daily. Evidence indicates that higher than currently recommended doses are necessary for effects in cancer, thus the current dose recommendations are heavily debated and expected to increase in the near future. According to the Food and Nutrition Board, daily administration of 2400 IU is NOAEL for vitamin D and it is considered safe without significant adverse events [Hathcock 2007]. Higher than currently recommended maintenance vitamin D doses are currently used in certain disease conditions. For example, replacement doses for osteomalacia and nutritional vitamin D deficiency are 50,000 IU weekly (equivalent to over 7,000 IU daily).

Major side effects are related to hypercalcemia, and clinical manifestations include polydipsia, polyuria, nausa, vomiting, abdominal pain, pancreatitis, headache, irritability, somnolence, and renal stone formation. Vitamin D toxicity will occur at serum 25(OH)D levels above 100 ng/ml (250 nmol/L), which would require a continuing oral intake in excess of 10,000 IU daily [Heaney 2005], which is much higher than the doses used in this trial. Thus, we do not expect to encounted these complication using the study doses. Concurrent high calcium intake along with vitamin D can lead to hypercalcemia, hypercalciuria and increased risk of renal stone formation [Jackson 2006]. Elevated urine calcium levels are a sensitive indicator of the increased risk of renal stone formation. Thus, in the current study, to avoid increased renal stone formation, we will 1) use safe doses of vitamin D; 2) will avoid excessive calcium intake; and 3) advise subjects to remain well hydrated and will carefully monitor urinary calcium excretion.

5.2 Availability

Vitamin D3 (cholecalciferol) tablets will be purchased from Vital Nutrients.

5.3 Agent Ordering

Vitamin D will be packaged in capsules of identical appearance, containing 800 IU, or 2400 IU per capsule. All capsules will be manufactured from the same lot of vitamin D3.

Vital Nutrients
45 Kenneth Dooley Drive, Middletown, CT 06457
Phone 860/638-3675 (888/328-9992), Fax 888/328-9993
http://www.vitalnutrients.net/vn.asp

5.4 Agent Accountability

Vitamin D3 tablets will be shipped and kept at the pharmacy in locked cabinets and mailed to study participants every 3 months. Study subjects will return their pill containers with the leftover capsules at every study visit.

6. DOSING DELAYS/DOSE MODIFICATIONS

Subject in the control group will take 800 IU vitamin D, while subjects in the experimental group will take 2400 IU daily. The 800 IU dose is standard of care, and the 2400 IU dose is the NOAEL dose for vitamin D. Thus, the study drug is expected to be well tolerated. There will be no dose delays or dose modifications in this trial. Subjects with severe symptomatic hypercalcemia will stop taking the study drug and will be withdrawn from the study. Severe symptomatic hypercalcemia is defined as a CTCAE grade 3 or higher hypercalcemia (albumin adjusted serum calcium above 12.5 mg/dL).

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

Major side effects are related to hypercalcemia, and clinical manifestations include polydipsia, polyuria, nausa, vomiting, abdominal pain, pancreatitis, headache, irritability, somnolence, and renal stone formation. Vitamin D toxicity will occur at serum 25(OH)D levels above 100 ng/ml (250 nmol/L), which would require a continuing oral intake in excess of 10,000 IU daily [Heaney 2005], 4-times higher than the planned maximum dose in this trial. Thus, serious adverse events are not expected to develop with the proposed vitamin D doses (maximum 2400 IU daily).

Concurrent high calcium intake along with vitamin D can lead to hypercalcemia, hypercalciuria and increased risk of renal stone formation [Jackson 2006]. Elevated urine calcium levels are a sensitive indicator of the increased risk of renal stone formation. Thus, in the current study, to avoid increased renal stone formation, we will 1) use safe doses of vitamin D; 2) will avoid excessive calcium intake (maximum daily intake of 1000 mg, dietary and supplements combined); and 3) carefully monitor serum calcium and urinary calcium excretion. Thus, with the currently proposed vitamin D doses and safety measures, renal stone formation is not expected.

7.2 Adverse Event Reporting

In order to protect subjects against or minimize potential risks, we will implement the special precautions of frequent monitoring of corrected serum calcium level and urinary calcium excretion as an indicator of vitamin D toxicity and risk of renal stone formation, and gradual increases of vitamin D doses, as outlined above. In addition, subjects will undergo a thorough medical assessment before enrollment, as well as repeated blood and urine analysis. Upon notification or detection of an adverse event that requires medical or professional intervention, research personnel will assist the subjects in obtaining said

intervention.

Data safety and monitoring plan: The study will be monitored by the Stanford Cancer Center, a Medical Monitor, the IRB and the HRPO. The Medical Monitor, James Ford, M.D., Associate Professor of Medicine, Pediatrics and Genetics, Stanford University, will be assigned to this protocol to monitor adverse events. At each study visit, patients will meet with the study physician to assess adverse events and determine causality. Events will be assessed whether they are unexpected and related to the research activity and harmful. An adverse event is any undesirable experience associated with the use of a medical product in a patient. The event is serious and will be reported when the outcome is: death, life threatening hospitalization (initial or prolonged), disability or requires intervention to prevent permanent impairment or damage. All serious adverse events will be reported, as required according to the FDA, NIH and Stanford IRB guidelines. The PD will review all adverse events and unanticipated problems as they arise. AEs will be reported using the Adverse Event Communication Form. AEs will be coded using MEDRA coding with CTCAE grading. AE's will be reported to the HRPO and the CCTO Safety Coordinator within 10 working days (5 days if the event is life threatening or resulted in death). Unanticipated adverse events will be reported to the FDA, HRPO and CCTO within 10 working days. If the sponsor determines that the unanticipated adverse event presents an unreasonable risk to the subjects, the study will be terminated as soon as possible, but no later than 5 working days after the sponsor makes the determination and no later than 15 working days after first receiving notification of the effect.

The PD will be responsible for all communication with the IRB, which will occur on an ongoing basis. All expected and non-serious AEs will be reported to the IRB during annual continuing renewal. *"All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study should be promptly reported by phone (301-619-2165), by email (hsrrb@amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RPH, 504 Scott Street, Fort Detrick, Maryland 21702-5012." "The medical monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death must be promptly forwarded to the USAMRMC ORP HRPO."*

Protocol deviations: Deviations from this protocol that fit the category of "unanticipated

problems involving risks to volunteers or others" or deviations that affect the scientific integrity of the study will be reported to the local IRB and the HSRRB within 24 hours." "Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the USAMRMC ORP HRPO as soon as the deviation is identified."

8. CORRELATIVE/SPECIAL STUDIES

There are no correlative studies included in this protocol.

9. STUDY CALENDAR

	Pre-Study	Mo 0	Mo 2	Mo 3	Mo 4	Mo 5	Mo 6	Mo 7	Mo 8	Mo 9	Mo 10	Mo 11	Mo 12	Off Study[c]
Investigational Agent		X	X	X	X	X	X	X	X	X	X	X	X	
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X----------X												
Vital signs	X	X			X				X				X	
Height	X													
Weight	X												X	
Performance status	X												X	
Serum and urine chemistry[b]	X	X			X				X				X	
Bone Mineral Density	X												X	
Adverse event evaluation		X ----------X												

a: *Investigational Agent*: Vitamin D (2400 IU in the experimental group, 800 IU in the control group)

b: Pre-Study and conclusion of study: serum calcium, phosphorus, creatinine, albumin, 25-hydroxy vitamin D (25(OH)D), parathyroid hormone (PTH), bone-specific alkaline phosphatase and urine spot calcium/creatinine ratio (fasting) and N-telopeptide (NTX) (second morning specimen); Month 4 and 8 visits: serum calcium, albumin, 25(OH)D (only mo 4), bone-specific alkaline phosphatase (only mo 4), urine spot calcium/creatinine ratio (fasting) and urine N-telopeptide (only mo 4)

10. MEASUREMENT OF EFFECT

10.1 Safety and efficacy

Efficacy will be evaluated using BMD T-score at 12 months, with comparisons to baseline and between groups. Safety will be evaluated on a 4-monthly basis with serum and urine calcium measures. AI-induced arthralgias and myalgias will be evaluated on a 4-monthly basis as well.

10.1.1 Definitions

Patients will be evaluated for toxicity on an ongoing basis (adverse events

reporting), as well as will be evaluated 4-monthly using serum calcium and spot urine calcium/creatinine ratio to monitor vitamin D toxicity. Efficacy will be evaluated via change in BMD measurement at the end of the trial (1 year) and compared to baseline (two measurements 1 year apart).

10.1.2 **Disease Parameters**

Efficacy will be evaluated with outcome measures such as change in spine BMD (primary outcome), markers of bone turnover (NTX, bone-specific alkaline phosphatase [BALP]), arthralgias and myalgias. Safety (secondary endpoint) will be evaluated by serum calcium and urine spot calcium/creatinine ratio.

10.1.4 **Response Criteria**

10.1.4.1 **Evaluation of Response**

Efficacy for treatment of AI-associated bone loss will be evaluated at the end of the treatment period (1 year). Secondary endpoints will be evaluated on a 4-monthly basis. Complete response will be defined as normal serum and urine calcium levels with normal bone turnover markers and significant reduction of bone loss with vitamin D treatment, and no myalgias/arthralgias. Partial response will be defined as normal serum and urine calcium with incomplete suppression of bone turnover markers and/or myalgias/arthralgias.

10.1.5 **Duration of Response**

It is expected that bone turnover markers will be elevated and arthralgias and myalgias develop shortly after starting AI therapy in the control group. We expect to see suppression of bone turnover and a decrease in arthralgias/myalgias in the experimental group. Considering that the primary endpoint is a change in BMD T-score at one year compared to baseline, efficacy parameters will not be evaluated before the conclusion of the trial. Safety parameters will be monitored on a bi-monthly basis.

11. DATA REPORTING / REGULATORY CONSIDERATIONS

11.1 **Monitoring plan**

This study will be monitored by the Data and Safety Monitoring Committee (DSMC) on a monthly basis.

11.2**. Stopping rules (for the individual patient and for the study as a whole)**

A study subject may end study participation at any time.

The Protocol Director will end the experiment for the individual study

subject if one of the following criteria is fulfilled:

- Adverse event or adverse effect, severe enough to justify the termination of study participation, as determined by the Protocol Director;
- Withdrawal of consent.

11.3 **Data management**

Demographic information and clinical data will be collected on case report forms (CRF), which will be stored in locked cabinets. This study data will then be stored in a password-protected database on a physically secure machine, which is back up nightly onto a HIPAA compliant server. This data will be validated and analyzed using SAS 9.1.3 with service pack 4 or later. Adverse events will be coded using an extensive dictionary and written notes will be kept at minimum for easier data entry. All changes made to the database, after initial entry, will be tracked. Every discrepancy between CRF and the database as well as inconsistencies within the database the will be tracked as well. Missing values will be identified in a regular basis and efforts will be made to obtain the missing values. Range checks will be performed at the time of data entry. “Self evident corrections” allowed to be fixed without review, will be tracked as well. Interim data checks (without interim data analysis) will be performed on a regular basis. Drs. Balint and Carlson will have equal access to the data. All PHI will be destroyed at the conclusion of the study. All data will be de-identified, subjects will be given a code and subject's names will be only known to the study doctors and their staff. For publication and analysis, data will be de-identified and normalized database will be used. De-identification of the BMD images data entails the removal of any PHI from the imaging headers. The information collected in regard to this study will be kept confidential to the extent provided by federal, state and local law. Access to and photocopying of the data collected with regard to subject participation in the study will be limited to the study doctors and their staff, the institutional review board, the Office for Human Research Protections in the U.S. Department of Health and Human Services, the U S Army Medical Research and Materiel Command (USAMRMC) and the United States Food and Drug Administration (FDA).

11.4 **Confidentiality**

Research staff will be all HIPAA trained and therefore knowledgeable about confidentiality. All data will be de-identified and subjects will be given a code. Study data and identifiers will be kept in separate files on separate password-protected secure computers and in locked cabinets in separate locations. Any hardcopy back-ups of the data will be stored in locked cabinets. All PHI will be destroyed at the conclusion of the study.

12. STATISTICAL CONSIDERATIONS

12.1 Endpoints

12.1.1 Primary endpoint

BMD spine T score, mean change from baseline at 1 year

12.1.2 Secondary endpoints

- BMD total hip T score, mean change from baseline at 1 year
- Proportion of subject with spine and/or hip BMD below -2.0 (clinically important loss of BMD)
- Proportion of subjects with arthralgias and myalgias as reported adverse events over time
- Urine NTx and BALP profile over time
- Serum 25(OH)D level achieved with vitamin D administration
- Proportion of subjects with elevated albumin-adjusted serum calcium or elevated fasting spot urine calcium/creatinine ratio

12.2 Analysis Populations

Efficacy analysis will be performed on the entire study population (intent-to-treat principle). We expect to see rapid bone loss in subjects who switch from SERMs to AI. The study is not powered to perform stratified analysis based on SERM exposure, but stratified randomization based on SERM exposure status will be performed to ensure balanced distribution between the groups.

12.3 Plan of Analysis

12.3.1 Background and Demographic Characteristics

Subjects with breast cancer (women only)
American Indian 0%
Asian 11.9%
Black 2.4%
Hispanic 7.7%
White 78.0%
Other 0%
Total 100%
Source: Stanford Health Services, Palo Alto, CA, 1995 patient census data
http://clinicalresearch.stanford.edu/Demographics.htm

12.3.2 Evaluation of Efficacy

To evaluate efficacy, we will determine whether NOAEL dose of vitamin D will be effective to treat AI-induced bone loss and high bone turnover, as measured by change in BMD T-score and bone turnover markers after one year of exposure to AIs. To assess efficacy, comparisons will be made

between treatment group assignment and serum vitamin D levels as well as end points related to bone turnover (change in urine NTx, serum alkaline phosphatase, and myalgias/arthralgias and fracture risk FRAX score). Considering that vitamin D is synthesized in the human skin after sun exposure which also contributes to 25(OH)D serum levels in addition to vitamin D3 supplements, the relationship between vitamin D doses, 25(OH)D levels and BMD will be further characterized using instrumental variables comparison analysis. Linear mixed effects analysis will be used to relate treatment group assignment to arthralgias/myalgias while allowing for intra-patient correlations.

12.3.3 **Methods for handling missing data and non-adherence to protocol:** A study subject may end the participation at any time. If subjects agree to be in the study and be randomized, they also agree to the final BMD testing at 12 months, regardless whether they decide to continue taking the study drug (drop in/out to study treatment). The study will continue enrollment until 100 subjects completed the first 4 months of follow-up, but no more than 120 subjects. The final analysis will include all study subjects who withdrew from the study (drop in/out to study treatment) or were excluded by the Protocol Director (intent-to-treat analysis). Complete case analysis will be performed. For missing data on the main outcome, multiple imputation based on a model that covaries on baseline variables will be used, in case data aren't Missing Completely at Random. For missing data for outcomes with several repeated measures, mixed effect models will be used. If drop-out/in to study treatment will exceed 20%, per protocol analysis will be performed as well. Patients will be assessed for outcomes as long as they consent to measurement, regardless of adherence to the treatment protocol, and the results analyzed by the intent-to-treat principle. Some patients may refuse follow-up measurements. When it is sensible to do so (i.e., when there is at least some post-baseline information to use) we will use multiple imputation as a sensitivity check on the 'all available data' ITT analyses. Since we assume a small proportion of missingness, and no association of missingness with treatment, we do not expect to see a substantial difference between the imputed and observed data [Lavori 1995].

12.3.4 **Evaluation of Conduct of trial (including accrual rates, data quality)** Data quality will be monitored on an ongoing basis to check for out-of-range and missing values and to make every effort to verify and correct them. Accrual rates will be monitored every 3 months and the study will be extended as necessary to include other facilities, in order to meet enrollment goals.

12.4 **Sample Size**

12.4.1 **Accrual estimates**

We expect to enroll about 50 subjects per year from Stanford Hospital and Clinics, for a total of 100 subjects within a 2-year period.

12.4.2 **Sample size justification**

a) We will determine whether NOAEL dose of vitamin D will be effective to treat AI-induced high bone turnover, as measured by change in BMD T-score and bone turnover markers after one year of exposure to AI. The primary outcome (change in spine BMD T-score, continuous outcome) will be analyzed, using analysis of covariance. The null hypothesis is that 2400 IU dose of vitamin D will not result in a significant difference in BMD at one year, compared to controls (800 IU, standard of care). We plan to enroll 100 patients, which powers the study for the efficacy outcome (primary outcome, change in spine BMD T-score). The dropout rate is estimated 15%. A 2.6 % reduction in spine and 1.7% reduction in hip BMD T-score have been reported in patients taking an aromatase inhibitor for 1 year [Eastell 2006, Dowsett 2005. With 50 subjects on each arm, the study has 80% power to detect a change that is 0.57 times the SD, and 90% power against 65% of SD. This is a medium size effect. Since no precise estimate of the SD is currently available, a rough estimate of about 3.0, based on the ATAC trial, is used [Eastell 2006]. Based on this SD estimate, a 57% SD change would translate to a 1.6% change in spine BMD. In the ATAC study, anastrozole caused a 2.6% drop, and tamoxifen lead to a 1.2% gain in hip BMD at 1 year, with a net difference of 3.8% between these two groups. Compared to tamoxifen, 2400 IU vitamin D is expected to result in a smaller fraction of change in BMD, but a clinically meaningful reduction in AI-induced bone loss.

For the secondary outcome, significant bone loss is expected to develop in up to 50% of subjects taking aromatase inhibitors for one year based on previous studies (see table below).

Outcome[a] Prevalence (%) in Vitamin D Treatment Group Needed to Detect[b] a Vitamin D Related Prevalence Reduction in a Trial with 50 Patients in each Arm.

Outcome[a] Prevalence (%) in Controls	Outcome Prevalence in Rx Group	
	80% Power	90% Power
25	5	3
30	8	7
40	14	12
50	23	17

b) The outcome is clinically important loss of BMD after one year of exposure to aromatase inhibitors.
c) With a two-tailed test of size $\alpha=0.05$

Most studies do not report hyercalcemia/hypercalciuria even with higher doses of vitamin D than this trial. If hypercalcemia develops in 4% or 6%, the probability is 80% and 91%, respectively that at least 1 out 40 patients will experience that event. Proportions (e.g. proportion of women with relief of joint pain) estimated on the basis of 40 patients carry a margin of error (95% confidence) of 16 percentage points at most.

12.4.3 **Criteria for future studies**

This protocol is not part of a sequence of trials.

12.5 **Interim analyses**

This is a small study, and interim analysis is not planned. Safety will be monitored in an on-going basis. NOAEL dose of vitamin D is considered safe, thus stopping the study for safety is not planned. Considering that the primary end point (BMD) will be only measured at 1 year for each subject and it is estimated that all the subject will be enrolled by the time efficacy data becomes available for the first 50 subjects, interim analysis will not be performed for efficacy either.

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APPENDICIES

A. **Participant Eligibility Checklist**

Study Institution #_____

Participant #_____

PARTICIPANT ELIGIBILITY CHECKLIST

_____*(Y)*	1. Does the patient have a histologically proven (from primary lesion and/or lymph nodes) diagnosis of invasive primary breast cancer?
(Y)	2. Was the primary therapy completed (surgical and/or chemotherapy?
(Y)	3. Is the patient a candidate to receive adjuvant aromatase inhibitor therapy?
(Y)	4. Does the patient have a life expectancy of at least 5 years?
(Y)	5. Does the patient have an ECOG performance status 0-2?
_____*(Y)*	6. Did the patient sign the informed consent?
_____*(Y)*	7. Is the patient postmenopausal: having had bilateral oophorectomy; aged more than 60 years; or aged 55-59 years with an intact uterus and amenorrheic for at least 12 months; or if amenorrheic for more than 12 months (after receiving hysterectomy, hormone therapy or chemotherapy)?
(Y)	8. Was bone mineral density measurement completed and the T-score is above -2.0?
_____*(Y)*	9. Is the patient a women, and at least 18 and no more than 85 years of age?
_____*(Y)*	10. Were the following lab parameters confirmed prior to study entry and within the normal range? Albumin adjusted serum calciu serum creatinine PTH fasting spot urine calcium/creatinine ratio >0.20
_____*(N)*	11. Does the patient take bisphosphonates, anticonvulsants, heparin, teriparatide or corticosteroids more then 5 mg/day?
_____*(N)*	12. Is the patient allergic to rice powder?
(N)	13. Is the patient pregnant or nursing?
_____*(N)*	14. Does the patient drink more than one drink a day?
	15. Does the patient have metastatic breast cancer High risk for osteoporosis/fractures (BMD < -2.0, history of non-traumatic fracture). Active hyperparathyroidism History of renal stones Diagnosis of stage 3 or higher chronic renal insufficiency Inability to absorb vitamin D due to an intestinal condition
Signed	_____
Print Name	_____
Dated	_____

Principal Investigator:

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For additional information about breast cancer research at Stanford Comprehensive Cancer Center please visit
http://cancer.stanford.edu

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Fax 650-498-4696

STANFORD
SCHOOL OF MEDICINE
Stanford University Medical Center

A Phase I/II randomized, double-blind, controlled study to evaluate efficacy and safety of vitamin D on bone mineral density and markers of bone resorption in aromatase inhibitor-induced bone loss in women with breast cancer.

Examining the effect of vitamin D on bone loss associated with aromatase inhibitors

A research project for women receiving therapy to reduce recurrence of breast cancer

Funded by
US Department of Defense,
Breast Cancer Research Program

Tel: 650-498-7977

How Can You Become Involved

We invite you to participate in our study of vitamin D and breast cancer. This study is funded by the US Department of Defense, Breast Cancer Research Program and is being conducted at Stanford University Medical Center.

Our Goals

The purpose of this study is to investigate the effect of vitamin D on bone loss associated with a group of medications called aromatase inhibitors, given for the prevention of breast cancer recurrence.

Benefits Of Participation

There may be no benefit to you from participating in this study. Your participation will provide us with valuable information that may be used to develop improved therapies for preventing breast cancer recurrence.

What Will Be Asked Of You

- Donate 1 tablespoon of blood and urine sample on 4 different occasions
- Have your bone density measured on 2 occasions
- Take vitamin D by mouth for one year
- That you allow us to access your medical record to collect information about your medical history and characteristics of your breast cancer.

Contact Information

If you are interested in participating or finding out more about this study please call:

Charlene Kranz
Project Research Coordinator
650-498-7977
Email: ckranz@stanford.edu

Who Is Eligible To Participate?

We are recruiting women who have had breast cancer and completed their primary surgical and/or chemotherapy and who are candidates to take medication called aromatase inhibitors to reduce recurrence of breast cancer.

Women are not eligible if they have severe bone loss (osteoporosis), have increased calcium or parathyroid hormone in their blood, have a history of kidney stones or have an intestinal problem that interferes with the absorption of vitamin D, or if they are known to be pregnant or are breast feeding.

For further information regarding questions, concerns or complaints about research, research related injury, and questions about the rights of research participants, please call (650)723-5244 or tool free 1-866-680-2906 or write the Administrative Panel on Human subjects in Medical Research, Administrative Panels Office, Stanford University, Stanford, CA 94305-5401